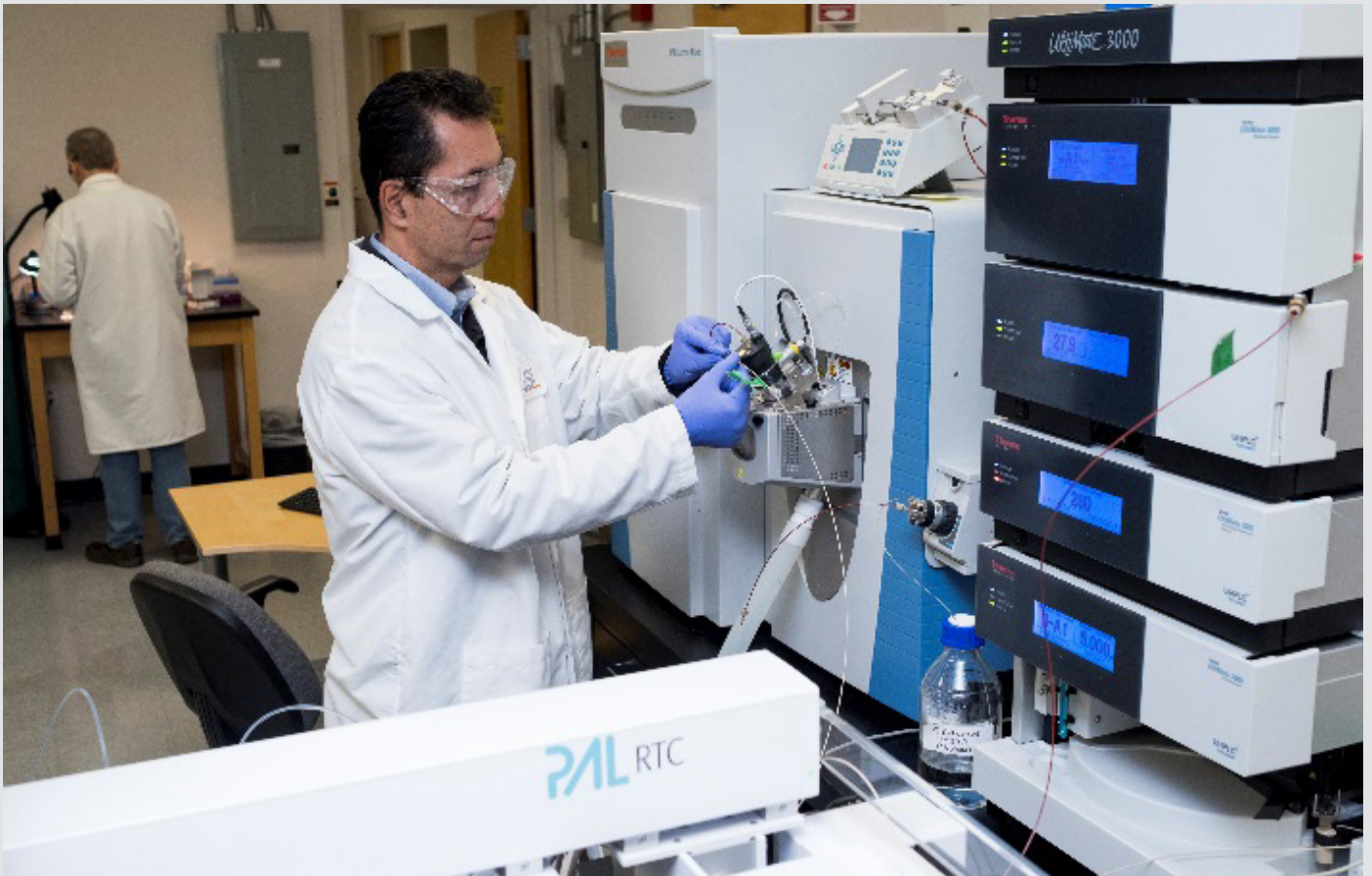


# SGS—Delivering excellence in protein structural characterization services

A case study in higher order structure characterization using hydrogen-deuterium exchange, liquid chromatography mass spectrometry

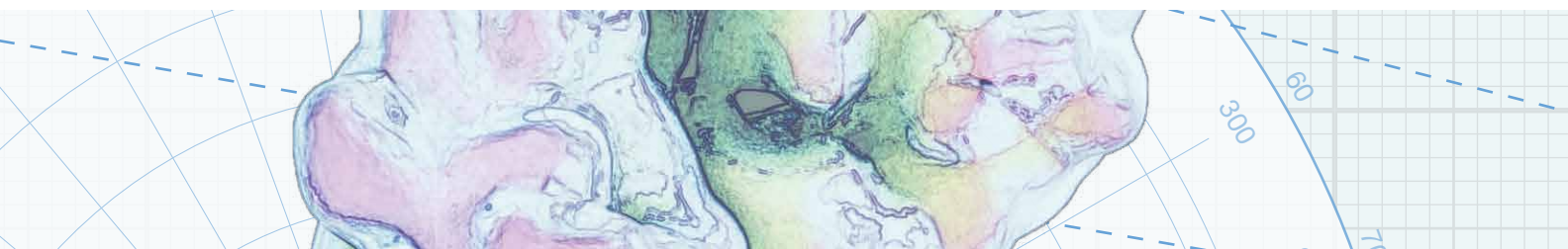
SGS is a world leading inspection, verification, testing and certification company. SGS has been a world leader in bio/pharmaceutical outsourcing for decades, partnering with companies of all sizes, including the top 20 pharmaceutical companies in the world.

SGS offers a full suite of protein characterization services to cGMP standards and is a global partner for clients looking to outsource their pharmaceutical and biologic product characterization services; having a network of laboratories with over 40 years of experience providing protein, peptide, biologic and biosimilar product analysis, their expertise is underpinned by combining cutting-edge techniques and technology, including a range of high performance mass spectrometers with industry leading knowledge.



“HDX-MS can characterize a protein a lot better than other biophysical characterization methods. I believe in the future of HDX-MS as a must-have in the protein characterization toolkit.”

—Yoshitomo Hamuro,  
HDX-MS Expert, SGS



With the ever increasing complexity of biotherapeutics being developed, comes the need to analyze these challenging products with analytical techniques that provide both increased levels of structural insights as well as confidence in the results obtained. One such technique that provides increased resolution, not information compared to traditional biophysical measurement techniques, and is a complete service offered by SGS, Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS).

SGS' clients typically have very complex problems that they wish to solve using HDX-MS, from the in-depth understanding of their drug product interaction sites to the characterization of the conformation of their protein drug itself, whether that is an antibody or other protein therapeutic.

The Thermo Scientific™ HDX-MS workflow allows SGS to identify surface sections of protein therapeutics which are open to hydrogen-deuterium exchange, which in turn can be used to provide information or to confirm its 3D structure. Depending upon the experiment performed, HDX-MS can:

- Facilitate the assessment of protein folding
- Determine protein conformation
- Identify conformational changes
- Provide information on protein-ligand binding interactions
- Map epitopes
- Compare biosimilars to innovator therapeutics

As illustrated within Figure 1, the Thermo Scientific HDX-MS workflow comprises of a comprehensive solution from sample preparation, separation, and data acquisition through to analysis; this system affords automated, robust and reliable protein labelling and digestion, followed by trap and elute chromatography, detection using High Resolution Accurate Mass (HRAM) Mass Spectrometry, and advanced peptide mapping and data visualization tools, all provided by:

- **LEAP Technologies H/D-X PAL™ Hydrogen Deuterium Exchange sampler system**

- Controlled by LEAP Technologies Chronos™ software and fully integrated with Thermo Scientific™ Xcalibur™ software

- **Thermo Scientific™ Dionex™ UltiMate™ NCS-3500RS system**

- Thermo Scientific™ Acclaim™ PepMap™ trapping column
- Thermo Scientific™ Hypersil GOLD™ C18 reversed phase column

- **Any Orbitrap-based LC-MS mass spectrometer, such as:**

- Thermo Scientific™ Q Exactive™ BioPharma MS platform
- Thermo Scientific™ Orbitrap Fusion™ Tribrid™ MS platform

- **Flexible data evaluation software**

- Thermo Scientific™ BioPharma Finder™ software
- Sierra Analytics HDExaminer™ software

The use of these selective, sensitive and robust analytical techniques are essential to ensure absolute confidence for the analysis of biotherapeutics, and the combination of both Leap Technologies and Thermo Scientific provides the most flexible HDX-MS system when it comes to delivering true structural insights.

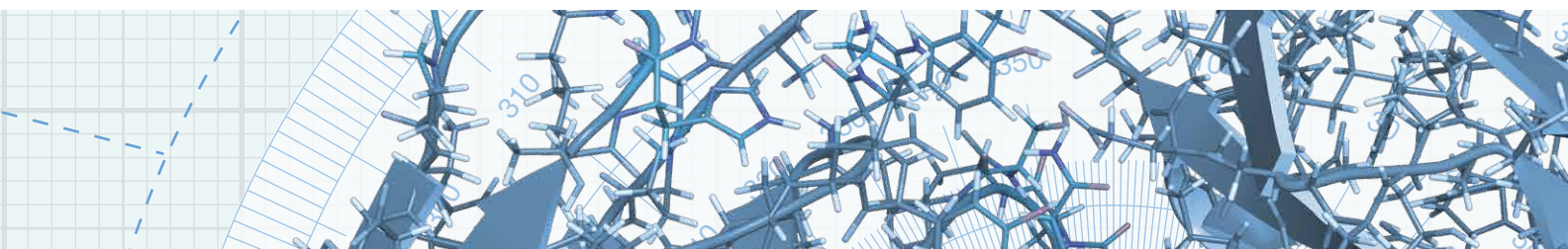
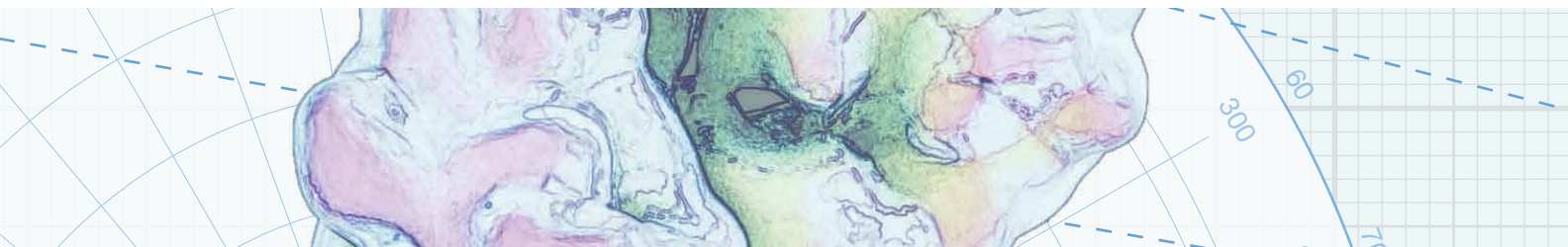
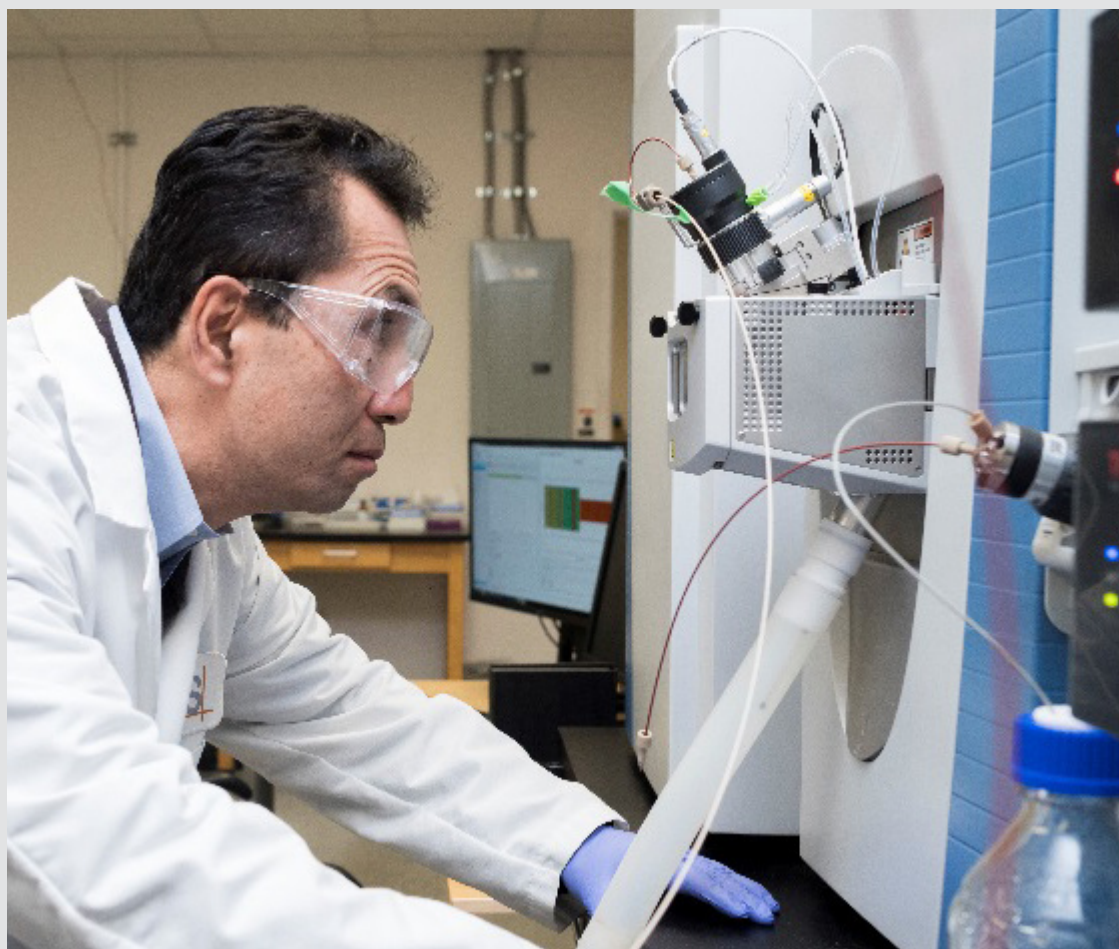




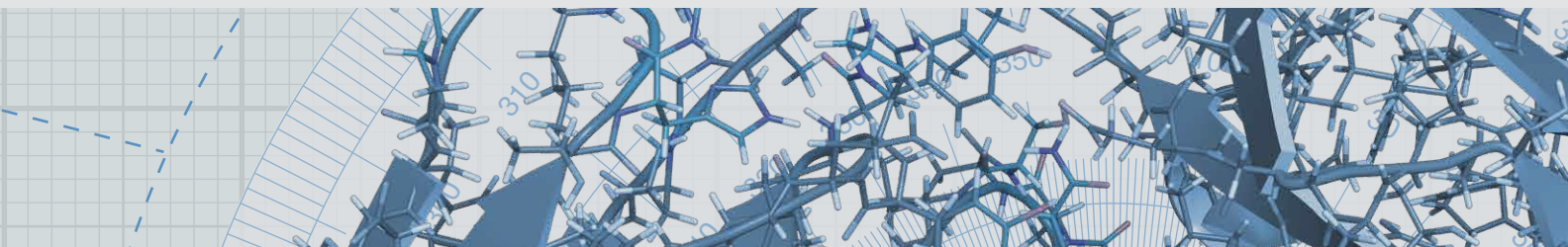
Figure 1. The Thermo Scientific HDX-MS workflow solution.





“The Thermo Scientific and Leap Technologies combination gives two advantages: the flexibility through the full editing capabilities of the Chronos software and its full integration with Xcalibur; and secondly, the resolution of the Q Exactive Orbitrap is higher than others combined with fast scanning which provides us with confident data.”

—Yoshitomo Hamuro,  
HDX-MS Expert, SGS





## The HDX-MS workflow solution

When evaluating their needs for HDX analyses, SGS considered the challenges that their sponsors would face, as well as their own internal requirements; to ensure that SGS could deliver timely results of high quality and accuracy, they required a system that not only provides flexibility for method development and future-proofing, but a system that was also easy to use when setting up, acquiring data and ultimately, processing the resulting information.

### Sample preparation

To ensure reliable results the sample preparation stage is critical. Using the H/D-X PAL auto-sampler allows sample preparation to be fully automated through the use of a single head with syringe exchange for syringes including one cooling syringe; this means that protein deuterium labelling can be performed at sub-ambient temperatures, along with in-line protein digestion and sample clean-up.

Importantly, the Chronos control software is fully integrated with Xcalibur, providing complete editing capabilities within a single control software environment.

### Separation

Once samples have been automatically prepared, they can be separated using trap and elute reversed phase chromatography with the NCS-3500RS using binary micro flow pumps, affording rapid separations. An additional ternary loading pump with flexible flow rate and solvent selection provides uninterrupted continuous flow through the trap and pepsin column, even during sample loading. To ensure absolute retention time stability, all components are housed within one enclosure with a fully thermostatted column compartment and switching valves.

### Data acquisition

Excellent sample preparation and robust separation demand the best when it comes to data quality. Orbitrap-based HRAM MS is the proven leader in peptide mapping due to its exceptional levels of mass accuracy, fast data acquisition rates, reliable and reproducible high resolution, all combined with multiple and advanced fragmentation techniques (such as Electron Transfer Dissociation, ETD) to provide the highest peptide identification rates and higher order structural elucidation.

## Data analysis

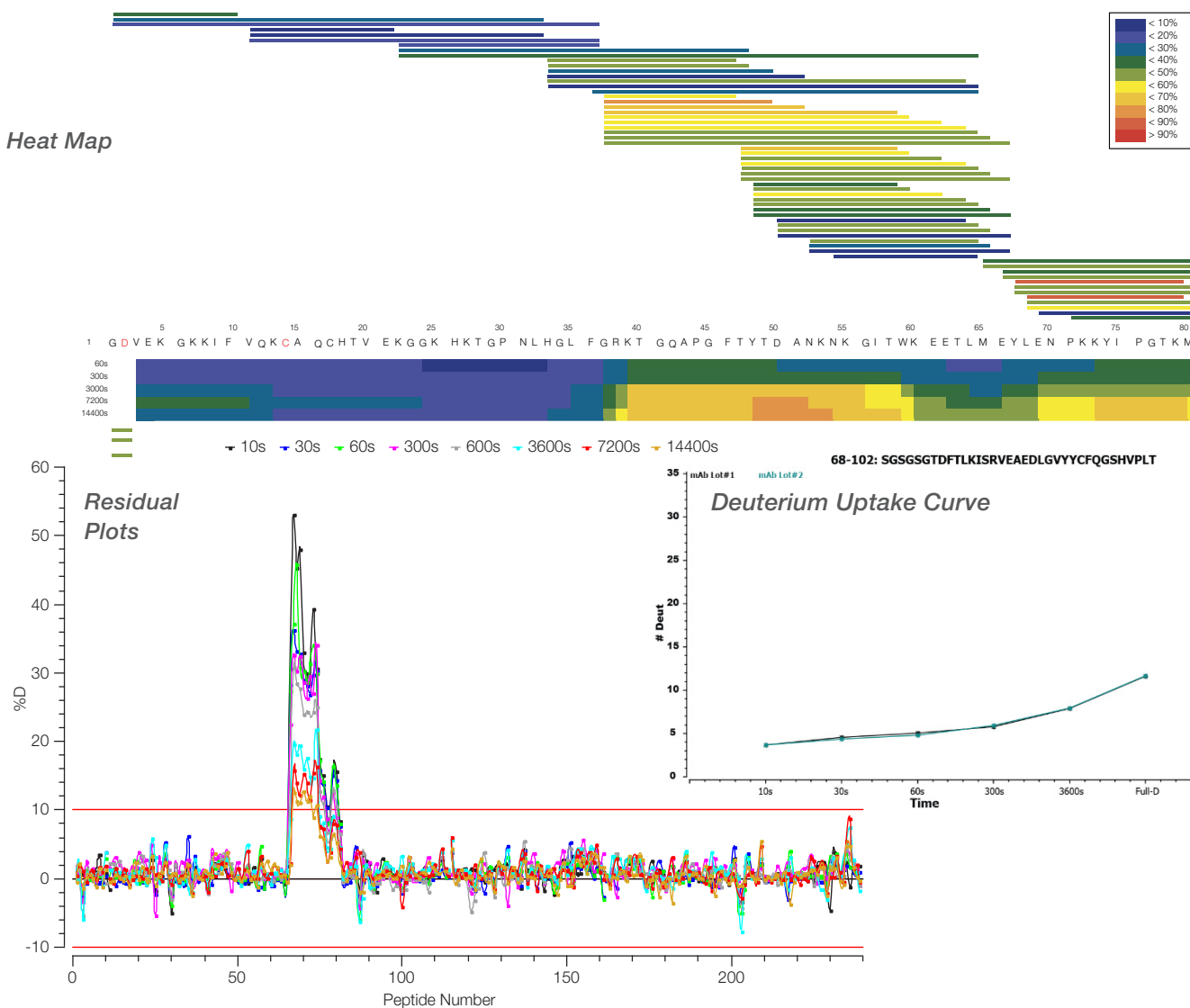
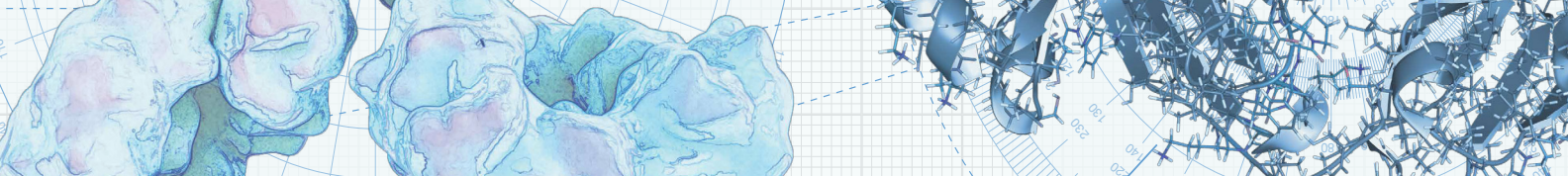


Figure 2. The combination of BioPharma Finder and HDExaminer software features provides a comprehensive set of data visualization tools to make the most complex of challenges easier to interpret.

Through the powerful combination of BioPharma Finder and HDExaminer software features, the high-quality data generated with the HDX-MS workflow solution can be quickly and easily interpreted, allowing SGS to provide a comprehensive array of results and answers to their sponsor's questions and challenges.

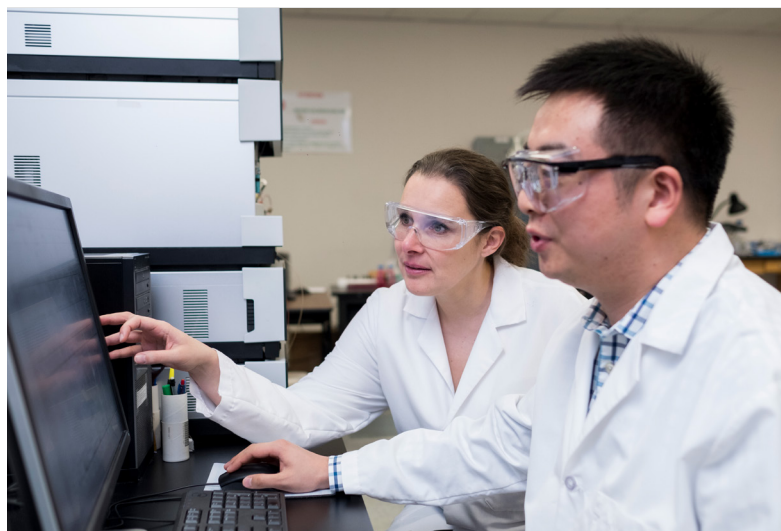
From the comparison of sequence residuals to identify differences between two experimental states, through to deuterium uptake plots, or more complex interpretation through heat maps and residual plots to name a few (as illustrated in Figure 2), this complete software solution for top-down/bottom-up HDX-MS data analysis provides a modern, flexible and high performance solution for industry and academia alike.



## A partnership beyond protein characterization

SGS are passionate about the high quality of services that they can provide to their clients, and embrace the role played by technology providers such as Thermo Fisher Scientific in helping them to deliver the quality that is expected. By working closely together, both SGS and Thermo Fisher Scientific look to lower costs, improve performance, increase functionality, all ultimately enabling drug manufacturers to bring life changing therapies to market more readily. These efforts allow us to play our part in making the world a healthier, cleaner and safer place to live.

For more information on SGS service for HDX-MS please visit [www.sgs.com/lifescience](http://www.sgs.com/lifescience)



“We feel like Thermo Fisher Scientific understands the agility requirements and pace of a CRO environment. They understand our challenges not just in buying, but also in maintaining and supporting equipment.”

—Dr. Bérangère Tissot,  
General Manager, West Chester, SGS

For more information on analytical technologies for extractables and leachables analysis, please visit [thermofisher.com/HDX](http://thermofisher.com/HDX)

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