

Characterizing Therapeutic Proteins for Drug Discovery and Development

A therapeutic protein mass spectrometry case study

LifeArc is the new name for MRC Technology, a medical research charity with a 25 year legacy of helping scientists and organizations turn their research into treatments and diagnostics for patients.

LifeArc is pioneering new ways to turn great science into greater patient impact. It brings together a network of partners to tackle specific diseases and directly funds academic and early stage research.

LifeArc's in-house expertise includes antibody development, small molecule drug discovery, diagnostics development and supports organizations with patent protection and licensing.

The charity works together with academics to help evaluate, fund and develop their research, it partners with other charities to increase impact and teams up with industry to medical research charities develop projects. To date these efforts have seen four life changing drugs successfully brought to market.



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These drugs include;

- **Vedolizumab** (ENTYVIO®, Takeda) a monoclonal antibody used for the treatment of ulcerative colitis and Crohn’s disease
- **Pembrolizumab** (KEYTRUDA®, Merck) a humanized antibody-based cancer immunotherapy agent
- **Tocilizumab** (RoActemra®, Roche) an immunosuppressive drug used in the treatment of arthritis
- **Natalizumab** (Tysabr®, Biogen) a humanized monoclonal antibody against the cell adhesion molecule α 4-integrin used for the treatment of multiple sclerosis and Crohn’s disease

LifeArc look to transform medical research

Building on its successful model of collaboration, LifeArc looks to bring together the best partners to drive medical innovation in specific therapeutic and diagnostic areas in response to unmet medical needs. LifeArc seeks to generate, fund and champion innovations in antimicrobials, neuroscience, personalized oncology and respiratory medicine.

To accommodate increased opportunities and a greater focus on collaboration, the Centre for Therapeutics Discovery moved to purpose-built laboratory facilities in Stevenage, UK in 2016. Here, over 80 scientists from across biology, chemistry and biotherapeutic work together with state-of-the-art equipment and capabilities.

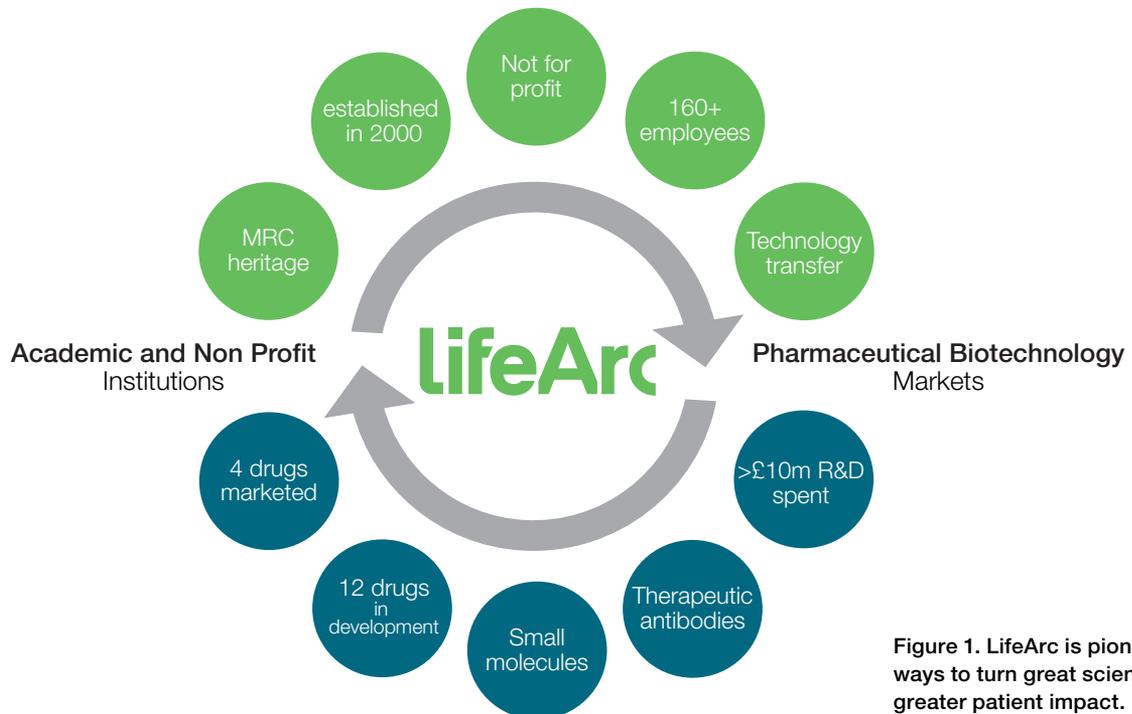


Figure 1. LifeArc is pioneering new ways to turn great science into greater patient impact.



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“Many of our academic collaborators have been using **Orbitrap technology** to aid their research for years, now it is great for us to apply it to both our biopharmaceutical and small molecule programs to accelerate their progression.”

—Dr. David Tickle,
Senior Principal Scientist, LifeArc

Driving protein-based drug discovery with mass spectrometry

Dr. David Tickle heads up one such team that provides a diverse range of therapeutic and target protein characterization expertise and services. Dr. Tickle and his group focus on protein-based drug discovery projects with candidate drugs coming from a number of sources, including academic collaborators, commercially sourced and in-house production. Traditionally the development of biologic and small molecule-based protein drugs is quality controlled via activity/efficacy binding assays. Unfortunately failure in these assays produces little additional information relating to cause and effect. Dr. Tickle describes his frustration with traditional methods: “If they fail we want to know what is happening and how do we make it better.”

Motivated by the need to learn more the team embarked on the utilization of high resolution accurate mass (HRAM) mass spectrometry (MS) techniques. Initially the group incorporated out-sourced MS analysis but found this approach lacked satisfactory response times and was expensive. The group decided they could save precious time and money by performing these assays in-house and as such decided to invest in a simple, yet flexible, mass spectrometry platform capable of performing a series of complimentary assays that would give detailed structural insight into candidate molecules; including native intact mass analysis, denatured intact mass analysis and peptide mapping.

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Complete biopharmaceutical characterization, on a single platform

The arrival of a new system in 2017 provided Dr. Tickle's group with all the necessary means to achieve full biopharmaceutical characterization together with additional scope for their existing small molecule (ADME) analysis workflows.

The system comprised:

- Thermo Scientific™ Vanquish™ Flex Binary UHPLC system
- Thermo Scientific™ Q Exactive™ Plus hybrid Quadrupole-Orbitrap mass spectrometer with BioPharma option
- Thermo Scientific™ BioPharma Finder™ software

The group had no previous experience with HRAM and hadn't analyzed a single protein before. Following initial familiarization training and information from the demo and online resources such as Planet Orbitrap the team were up and running with samples from day one.

The team can confidently use the instrument with its ease of use further demonstrated by the fact that the most recent industrial placement student was able to prepare, run and analyze samples for all characterization workflows within a week.

“The system gets tuned and calibrated once a week. It's **incredibly stable** and easy to set-up.”

—Dr. David Tickle,
Senior Principal Scientist, LifeArc



Figure 2. Q Exactive BioPharma platform with BioPharma Finder software—a complete solution for intact and bottom-up protein mass spectrometry.



“It’s as easy to use as any other instrument in our lab. We were up and running in days. Data analysis is quick and easy—I spend longer inputting the sequence!”

—Dr. David Tickle,
Senior Principal Scientist, LifeArc

More with your sample

The group receives small samples of candidate antibody and antibody-drug conjugate molecules from their collaborators; typically 10 μL of a concentration of 1 $\mu\text{g}/\mu\text{L}$. The sensitivity of the Q Exactive BioPharma platform allows LifeArc to do much more with each sample. The group routinely incorporate all three modes of data acquisition (high mass range, protein mode and standard mode) when characterizing each candidate to give a comprehensive overview of the structure:

Intact level

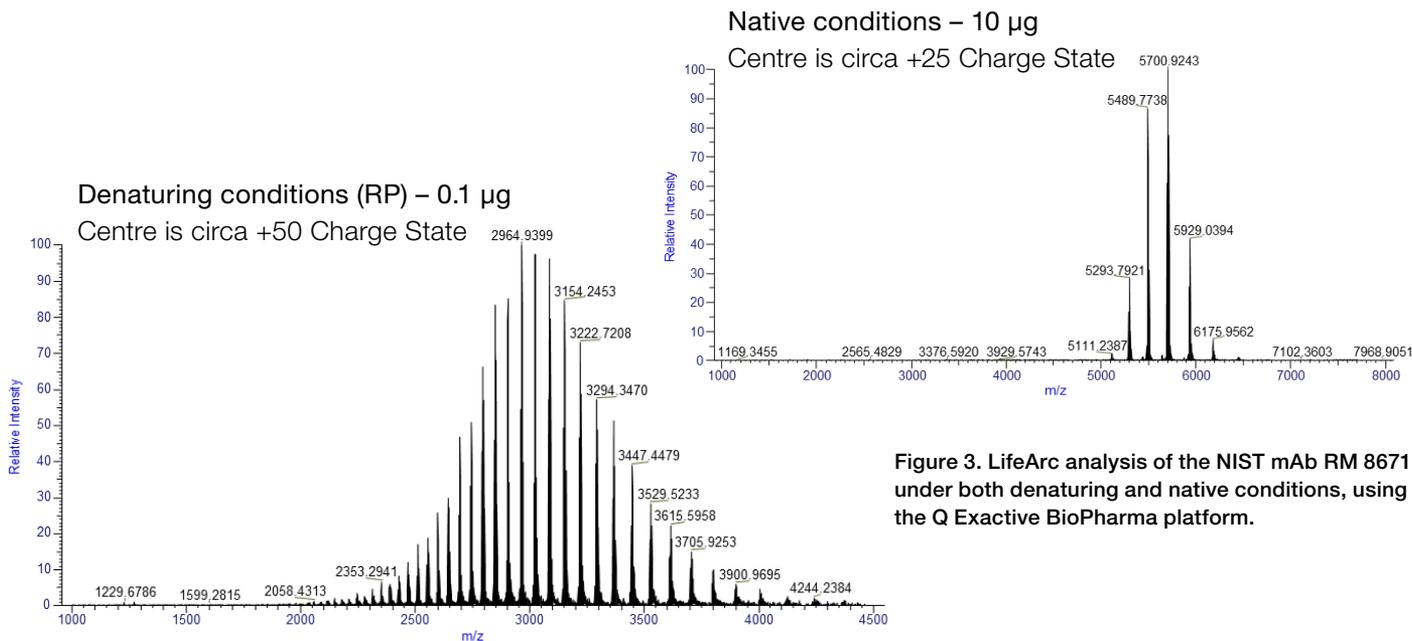
Typically the analysis will commence at the intact level, under either denaturing or native conditions. This takes advantage of the ‘high mass range’ (HMR) acquisition capabilities of Q Exactive BioPharma platform (with improved transmission and scanning up to m/z 8,000) and gives insight into molecular mass with detailed baseline resolution of the various isoforms, Figure 3. Native analysis simplifies the charge envelope and streamlines interpretation as it is easy to perform on the Q Exactive BioPharma platform due to robust mass accuracy, simple walk-up settings and efficient desolvation.

Sub-unit level

Intact mass analysis is often followed by sub-unit analysis, using ‘protein mode’. Here the team take advantage of the exceptional resolution and mass accuracy of the Q Exactive BioPharma platform which is able to deliver isotopic resolution of mAb sub-units, Figure 4; allowing some process modifications, such as deamidation, to be recorded even at the sub-unit level. Resolution of up to 140,000 (FWHM) at m/z 200 allows simplified matching of isotopic mass up to 25,000 Da and significantly simplifies deconvolution.

Peptide level

Finally, ‘standard mode’ is used for peptide mapping analysis which is called upon for specific follow-up when for instance PTM localization or specific sequence variant information is required.



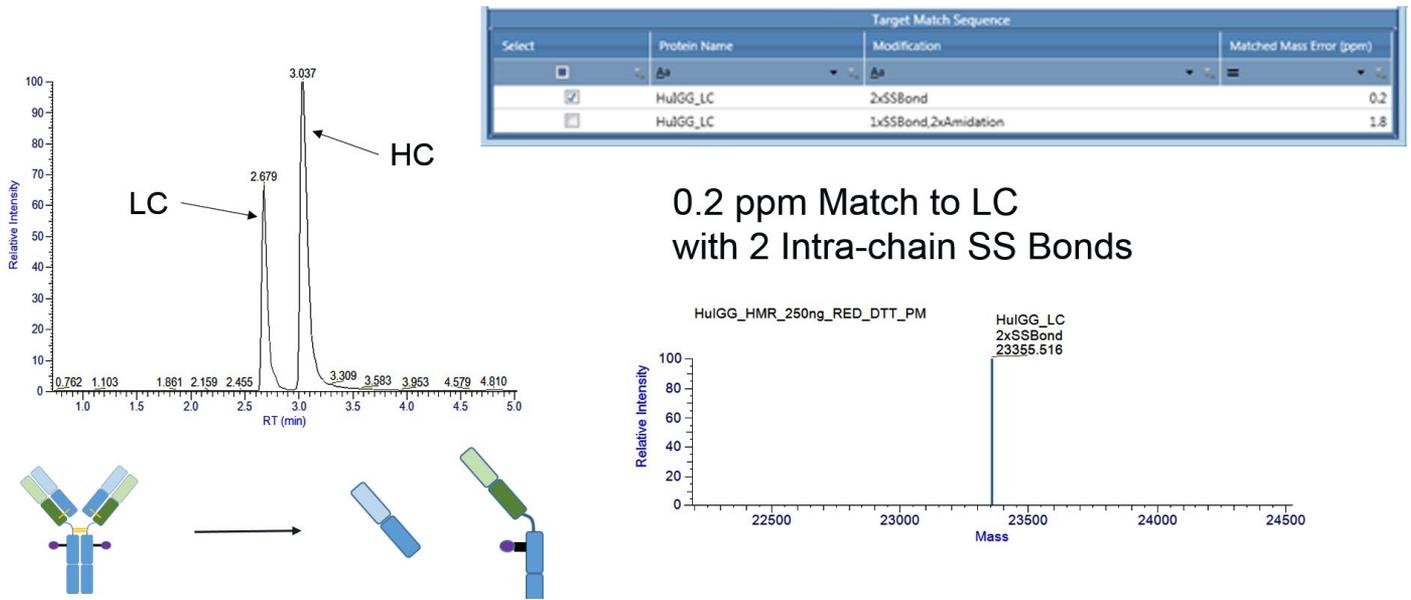


Figure 4. Analysis of humanized mouse IgG light chain sub-unit, using the Q Exactive BioPharma platform in protein mode.

Easily move from small to large molecules

In line with the pharmaceutical industry, the LifeArc team are experiencing a steady increase in protein-based therapeutic candidates. Dr. Tickle, with his background in analytical chemistry and small molecule pharmaceutical analysis, relishes these new challenges. Dr. Tickle believes the transition from small to large molecules is aided by a certain mind-set and by applying the same analytical approaches to both.

With the extremely high mass accuracy of the Q Exactive BioPharma platform you can be confident that any discrepancy is the result of real modifications which merit further characterization or investigation. Dr. Tickle and other members of his team apply proper experimental rigor to their analysis and for biopharma candidate analysis they incorporate the use of an antibody standard for method set-up and system suitability and stability testing, such as NISTmAb (Reference material RM 8671, NIST). Routine incorporation of these standard checks

have highlighted the impressive robustness and stability of the platform, something that Dr. Tickle was pleasantly surprised by, having expected more frequent tuning to be necessary to maintain such high levels of mass accuracy.

In addition to requiring less tuning than anticipated, Dr. Tickle was also surprised at the ease of which tuning and method optimization can be achieved. It is the first HRAM MS system at LifeArc; however, with the aid of standard parameters and useful resources provided within the [Planet Orbitrap](#) community, the team has been able to quickly optimize methods for the wide variety of candidates that come into the laboratory.

The team currently enjoy a suite of standard methods for each analysis mode on the platform and for different types of molecules.





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Simple acquisition under native conditions

Native mass spectrometry is an orthogonal way of looking at samples. Advanced desolvation and parameter standardization on the Q Exactive BioPharma platform make the acquisition of native spectra as easy as under denaturing conditions. It delivers increased spatial resolution between charge states and allows greater structural insights into the protein, including how the formation of non-covalent complexes.

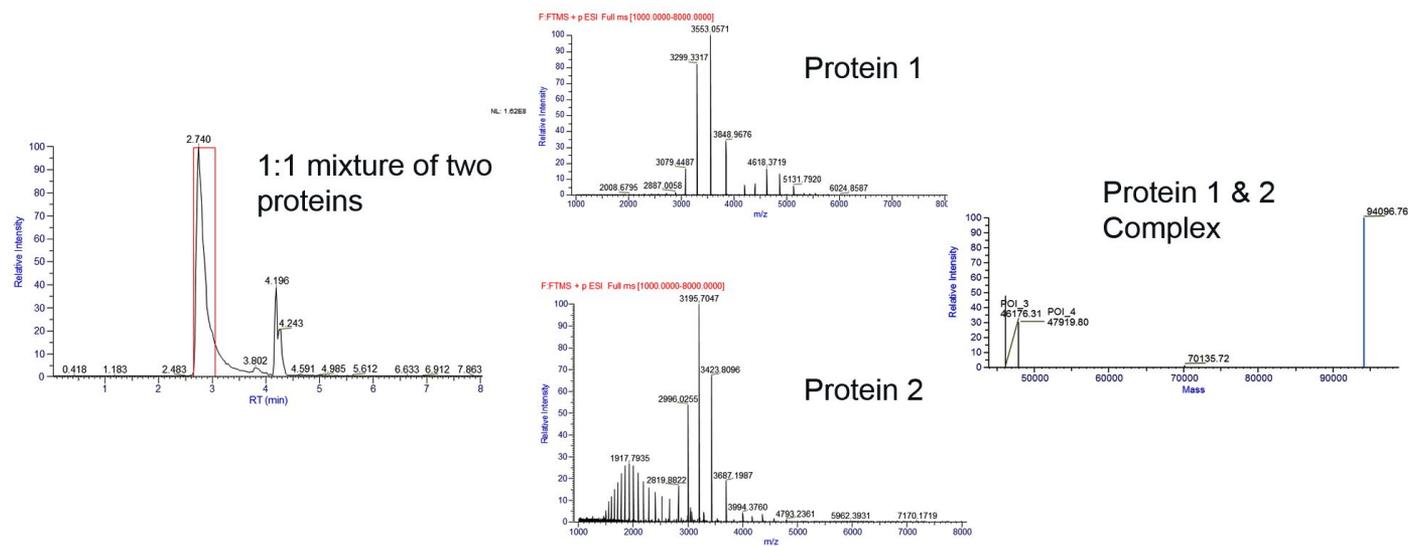
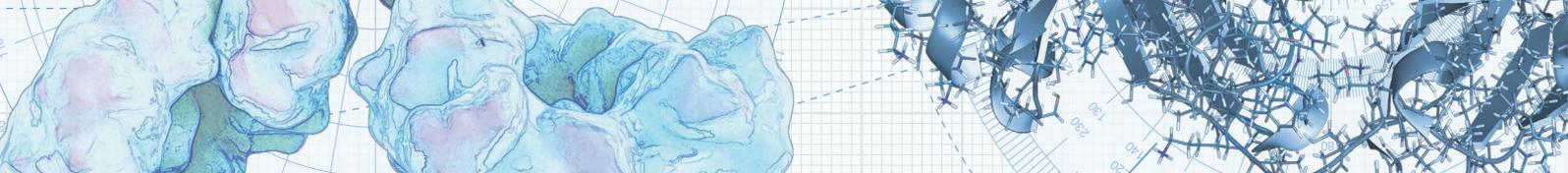


Figure 5. LifeArc study of protein:protein complex formation using SEC-MS and native-like conditions on the Q Exactive BioPharma platform.



Antibody drug conjugates (ADCs)

LifeArc has key expertise in drug conjugation strategies. They now incorporate HRAM MS to investigate how different conjugation conditions and chemistries can be varied to effect drug distribution. The high spatial resolution offered by the HMR mode on the Q Exactive BioPharma platform provides unrivaled structural insights and the most accurate information on ADC drug to antibody ratios (DAR). It is hoped that this information will prove valuable for their collaborators in the development of these more advanced biologics.

Conclusions

Dr. David Tickle and his team at LifeArc have taken full advantage of the key capabilities offered by the Q Exactive BioPharma platform and BioPharma Finder software. The seamless and rapid integration of this technology into their drug discovery workflows gave LifeArc the ability to provide their collaborators with the expertise and results necessary to accelerate the development of even their most advanced candidate drugs.

For more information on LifeArc please visit www.lifearc.org

Lysine conjugated ADC, DAR = 7.0

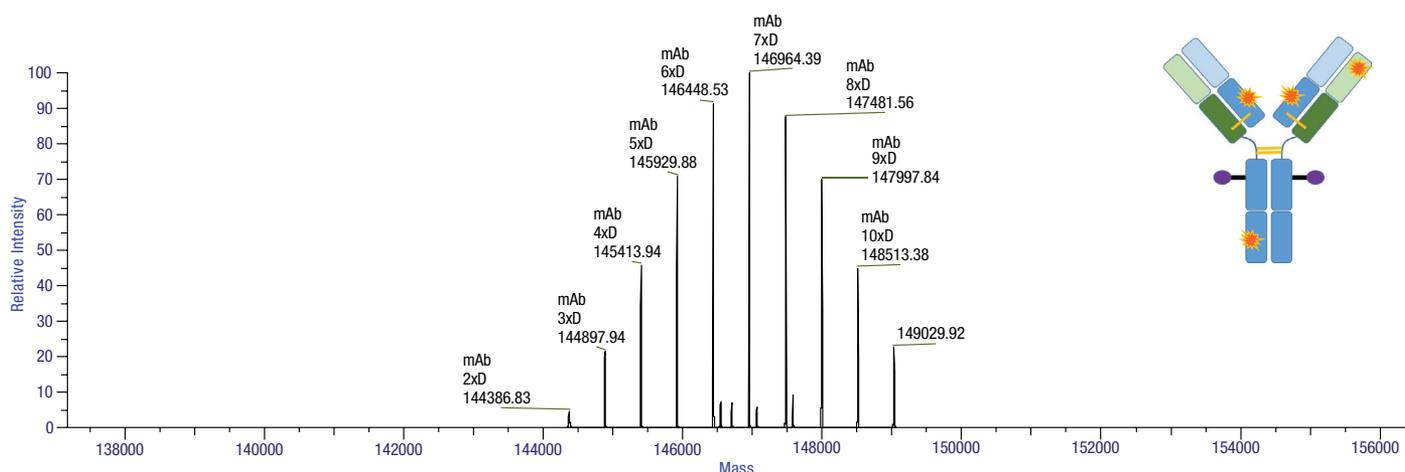


Figure 6. Analysis of experimental ADC after post-deglycosylation step.

For more information on the Q Exactive BioPharma platform, please visit thermofisher.com/QEBP