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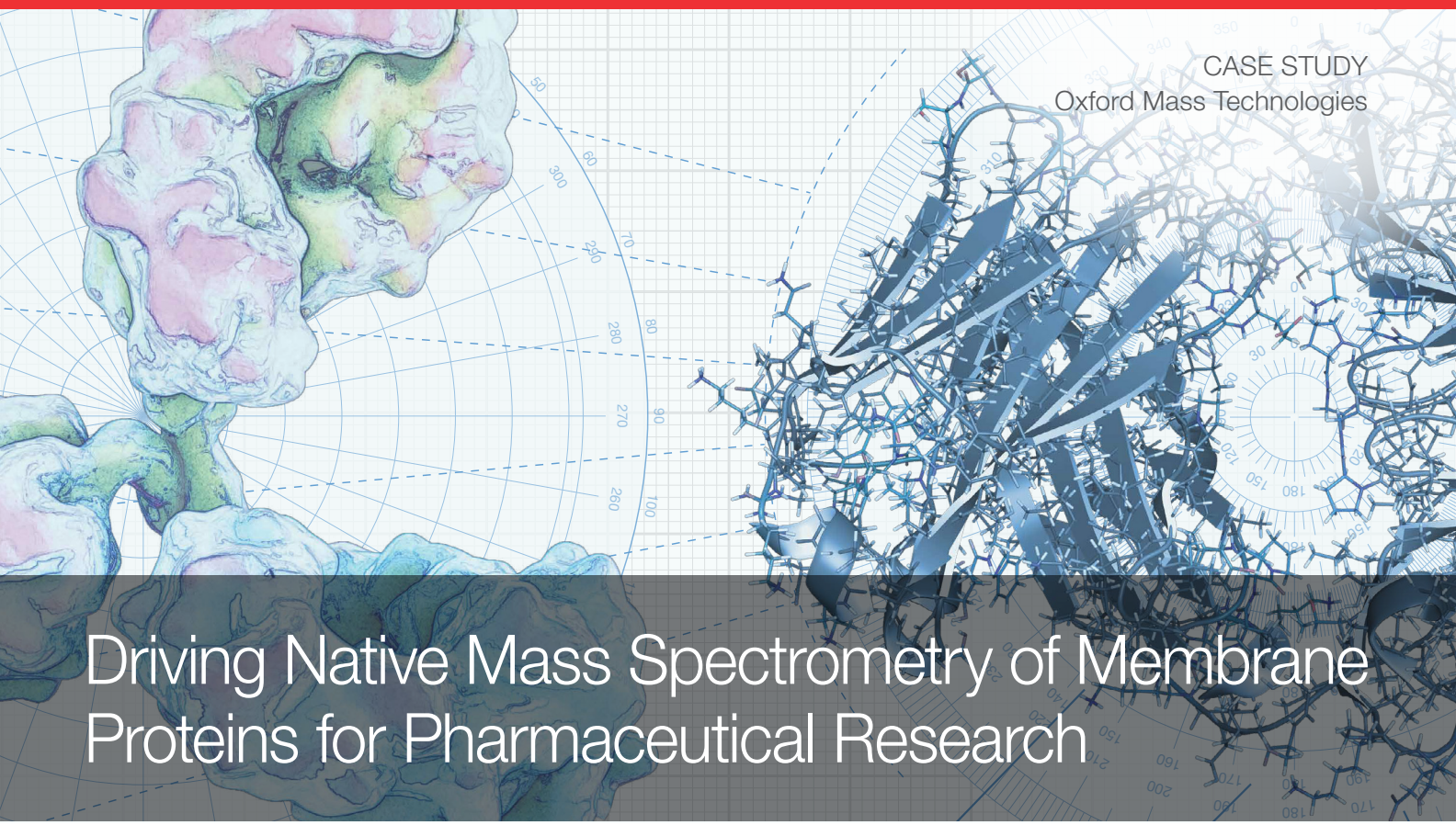

CASE STUDY
Oxford Mass Technologies

Driving Native Mass Spectrometry of Membrane Proteins for Pharmaceutical Research

New technology for advanced protein characterization

Oxford Mass Technologies (OMass) specializes in native mass spectrometry platforms for multidimensional analysis of challenging protein targets for the biopharmaceutical industry.

Unlike conventional proteomics-based mass spectrometry (MS), native MS relies on maintaining a biomolecule's folded state and any associated noncovalent interactions within the mass spectrometer. Native MS allows direct detection of proteins and protein-ligand complexes at unprecedented resolution with the Thermo Scientific™ Q Exactive™ UHMR (Ultra High Mass Range) system.

OMass is the first company to offer native MS of membrane proteins, the largest family of drug targets. Membrane proteins are largely hydrophobic molecules which require highly heterogeneous micelle assemblies for solubilization. This renders them extremely challenging for analysis by many biophysical methods, and they were

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long believed to be incompatible with native MS. OMass' platform is built on years of advancements in sample preparation and instrumentation towards tackling this challenge, with the use of carefully selected detergents and finely tuned gas-phase manipulation capabilities which allow sampling of membrane proteins directly from native-like environments. Its vision is to apply this exciting new technology to solve some of the most interesting questions faced in drug discovery.

Since the landmark publication of the first analysis of a membrane protein complex by native MS in 2008, the team behind OMass has been developing a toolbox of experimental know-how in studying these assemblies. After many academic collaborations with numerous pharmaceutical companies, OMass was created to satisfy the growing demand for these technologies.

"Membrane proteins represent an extremely challenging class of drug targets for all biophysical techniques but are of essential interest to current and future drug discovery."

—Professor Dame Carol Robinson,
Founder and Chief Scientific Consultant,
OMass Technologies

"Pharmaceutical research remains one of the most important challenges in modern science, which has a huge impact on the world around us. Applying native MS technologies to research in this field and working with some of the world's leading pharmaceutical companies is deeply rewarding."

—Dr. Jonathan Hopper, CEO,
OMass Technologies

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Applicability throughout the drug discovery pipeline

OMass' technology can augment protein production and characterization along various stages of drug development, including:

Protein purification—In early phase research, the production of membrane protein targets at suitable yield and purity can be a rate-determining step. OMass provide a high-resolution accurate mass (HRAM) analysis platform for assessing purification protocols. This facilitates optimization of crucial steps such as detergent screening and delipidation. Native MS provides a direct measure of protein homogeneity and stoichiometry, with high resolution also informing on the presence of lipids, impurities and truncated variants corresponding to different preparations, allowing identification of optimal conditions.

Binding quantification—During candidate characterization, it is often desirable to interrogate the binding affinities of ligands to proteins or protein complexes. Whether the target is a single protein or a large assembly, native MS can provide quantitative information on ligand binding by monitoring discrete changes in mass with relative signal intensities reporting on the entire population of solution binding equilibria, unlocking stoichiometries, affinities and allosteric effects.

Biotherapeutic analysis—In late stages of drug discovery, native MS is particularly valuable in the development of therapeutic antibodies. OMass' platform can rapidly assess sequence integrity, glycan profile and antigen binding activity in a single measurement using only picomoles of material. For antibody-drug conjugates (ADCs) and bispecific antibodies (bsAbs), native MS is useful in assessing drug-to-antibody ratio and composition.

The role of OMass is to provide availability of this cutting edge technology to research groups involved in the drug discovery process from early phase to late stage, and across a range of drug development programs. Pharmaceutical companies can thus capitalize on areas where native MS can either complement or enhance their research pipelines.

"With Orbitrap-based mass spectrometry technology we have been able to get deeper insight into extremely complex protein and ligand binding systems"

—Dr. Hsin-Yung Yen, CSO,
OMass Technologies

Staying up-to-date with the latest in native MS technology

To ensure access to the latest innovations enabling them to tackle challenges in membrane protein analysis, OMass maintains close collaborative relationships with key manufacturers of mass spectrometers. This has resulted in the development of bespoke systems including the Thermo Scientific™ Q Exactive™ UHMR (Ultra High Mass Range) instrument. This system has been used for analysis of ions with mass-to-charge ratios exceeding 40,000, opening the door to new insights into ever larger protein complexes under native conditions.

"Thermo Fisher has made a major contribution to native mass spectrometry in recent years. In the past we couldn't get resolution of very large protein complexes with few charges. This was a real stumbling block for us. We have benefited from recent developments in native high-resolution mass spectrometry. We didn't realize what we weren't seeing before. It gives us a new view of our molecules and this is an exciting transformation. I'm very excited where it will take us in the future."

—Professor Dame Carol Robinson,
Founder and Chief Scientific Consultant,
OMass Technologies

At the frontier of unraveling the protein-lipid landscape

In recent years, our understanding of interactions between proteins and lipids has evolved considerably. Rather than merely anchoring proteins in the membrane nonspecifically, lipids can interact in a targeted manner with proteins to directly modulate their structure and function. Recent studies published by Professor Robinson have demonstrated that native MS is uniquely suited to study the effects of protein-lipid binding. The ability to observe co-purified lipids, and even introduce defined endogenous lipids and resolve all binding populations simultaneously, allows interactions to be interrogated in terms of affinity or conferred structural stability.

These capabilities present distinct advantages when using native MS, as an to alternative techniques such as isothermal titration calorimetry (ITC) or surface plasmon resonance (SPR), which are more challenging for complex equilibria. The OMass team spent years developing native MS as a complementary approach to existing methods for studying extremely challenging systems. G-protein coupled receptors (GPCRs) and ion channels are two such protein classes that are highly sought after drug targets for a range of diseases, which have formed the basis for several collaborations with OMass.

These systems often possess complex interaction networks, consisting of cofactors or other biomolecules in addition to the candidate drug. In-depth characterization thus requires techniques that are able to sample multiple co-existing states simultaneously, with sufficient resolution to independently observe the populations of interest. These strengths of native MS have now been demonstrated in several research publications and find great application to the pharmaceutical industry, where increasingly complex protein systems have become targets of future therapeutic candidates.

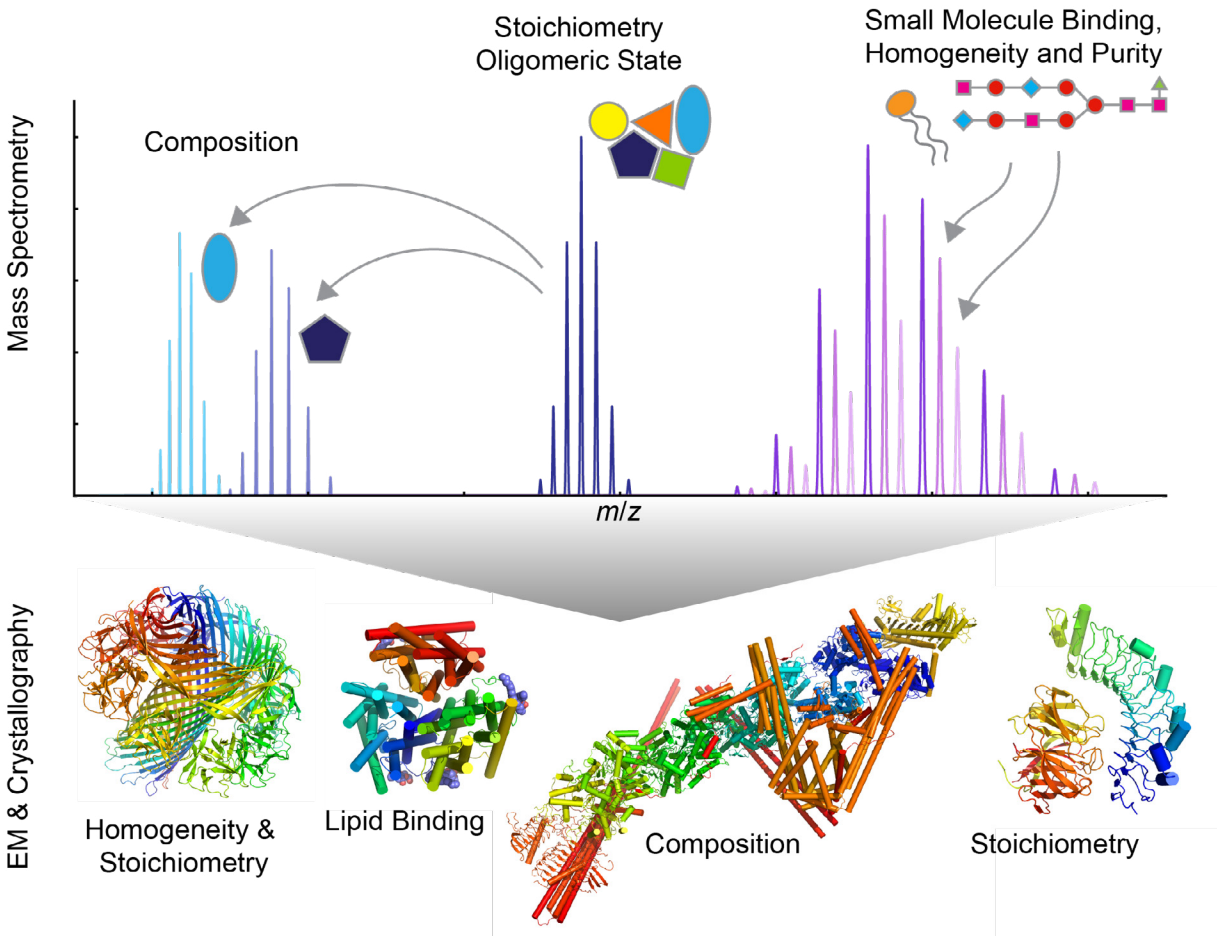

Figure 1. A representation of native MS data typically observed for a large, oligomeric protein complex. The accurate mass obtained defines the oligomeric state and stoichiometry of non-covalent complexes. Activation of complexes, for example by collision-induced dissociation, allows complexes to be dissociated into their constitute components, providing further details of composition. Small molecule binding with ligands such as lipids, substrates and drugs represent discrete increases in mass (adducts). The information obtained from high-resolution mass data is complementary to many other biophysical techniques, such as X-ray crystallography and electron microscopy, allowing for instance unknown electron densities to be attributed to specific molecules observed in native MS.

A new toolkit for biotherapeutics

Pharmaceutical research is increasingly focused on harnessing the superior specificity and efficacy of proteins themselves as therapeutic agents (biologics). Their complexity in contrast to small-molecule drugs presents new demands on analytical science to develop platforms capable of not only studying proteins in a pre-clinical R&D environment, but also to control the quality of resulting drug products. Evaluating and adapting native MS for this purpose is one of the primary goals at OMass. Direct mass measurement of folded antibodies from aqueous solutions provides insight into unwanted truncations, impurities, or other causes of heterogeneity.

Post-translational modifications are also monitored: for antibodies in particular, various glycoforms of the protein are resolved and their relative abundances quantified. Native MS presents several advantages over orthogonal methods of acquiring this information, including rapid and simultaneous acquisition (a single spectrum provides this information in minutes—seconds for certain throughput platforms) and very low sample requirements. Antibodies can be analyzed in the presence of the target receptor to confirm activity under the same conditions. OMass also offers hydrogen-deuterium exchange (HDX) as part of pre-clinical R&D to identify the region(s) of a receptor involved in binding a specific antibody, without the need for labor and cost-intensive structural techniques.

Using the latest in native mass spectrometry technology from Thermo Fisher Scientific, scientists at OMass are equipped to assist in tackling some of the most exciting challenges in drug discovery today. From membrane protein targets to biologics, the platform delivers confident characterizations of complex protein interactions at unmatched mass resolution. OMass looks forward to expanding their partnerships to augment pharmaceutical and biotechnology pipelines. For more information about mass spectrometry services offered by OMass please visit www.omasstech.com

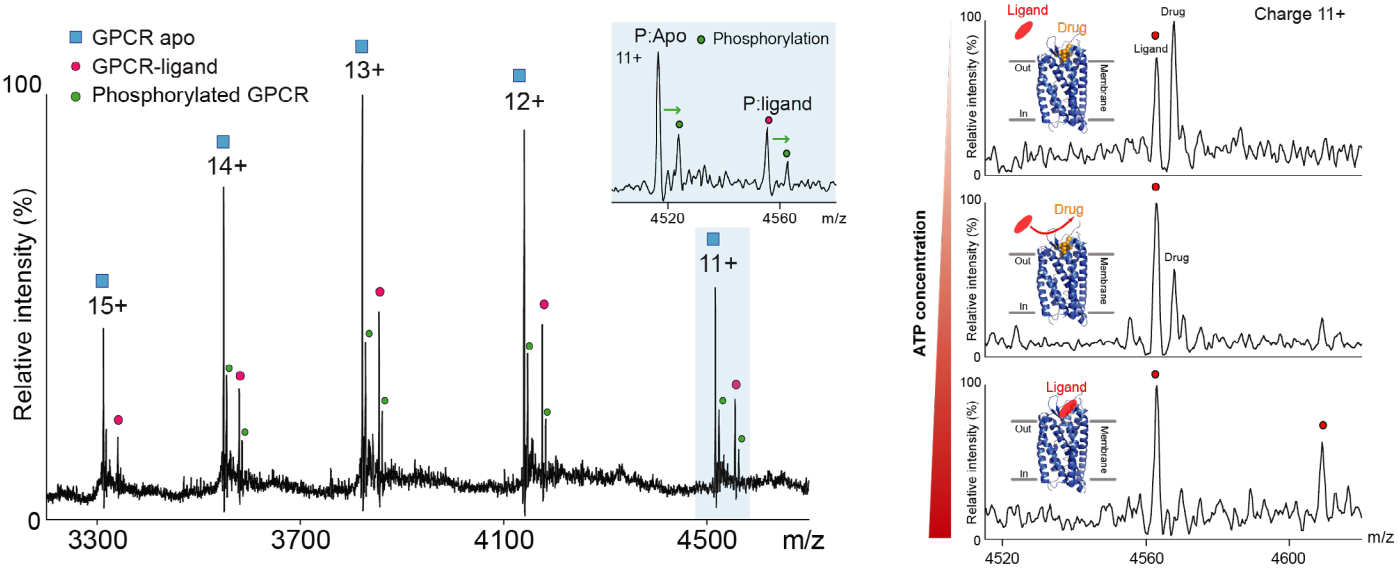

Figure 2. A native mass spectrum of the class A GPCR receptor P2Y1R, liberated from micelles. Distinct distributions allow the relative populations of unbound receptor and endogenous ADP:P2Y1R complex to be determined. Post-translational modifications, in this case a phosphorylation, are also observed and resolved. Incubating the receptor with a known drug allows the binding to be captured and used to study competition with increasing concentrations of a natural ligand.

"The Thermo Scientific Q Exactive UHMR mass spectrometer has been a game changer in native mass spectrometry. Due to the very high resolution we can now measure small molecules bound to large proteins, which is an important step forward for fragment-based drug discovery."

—Dr. Idlir Liko, CTO, OMass Technologies

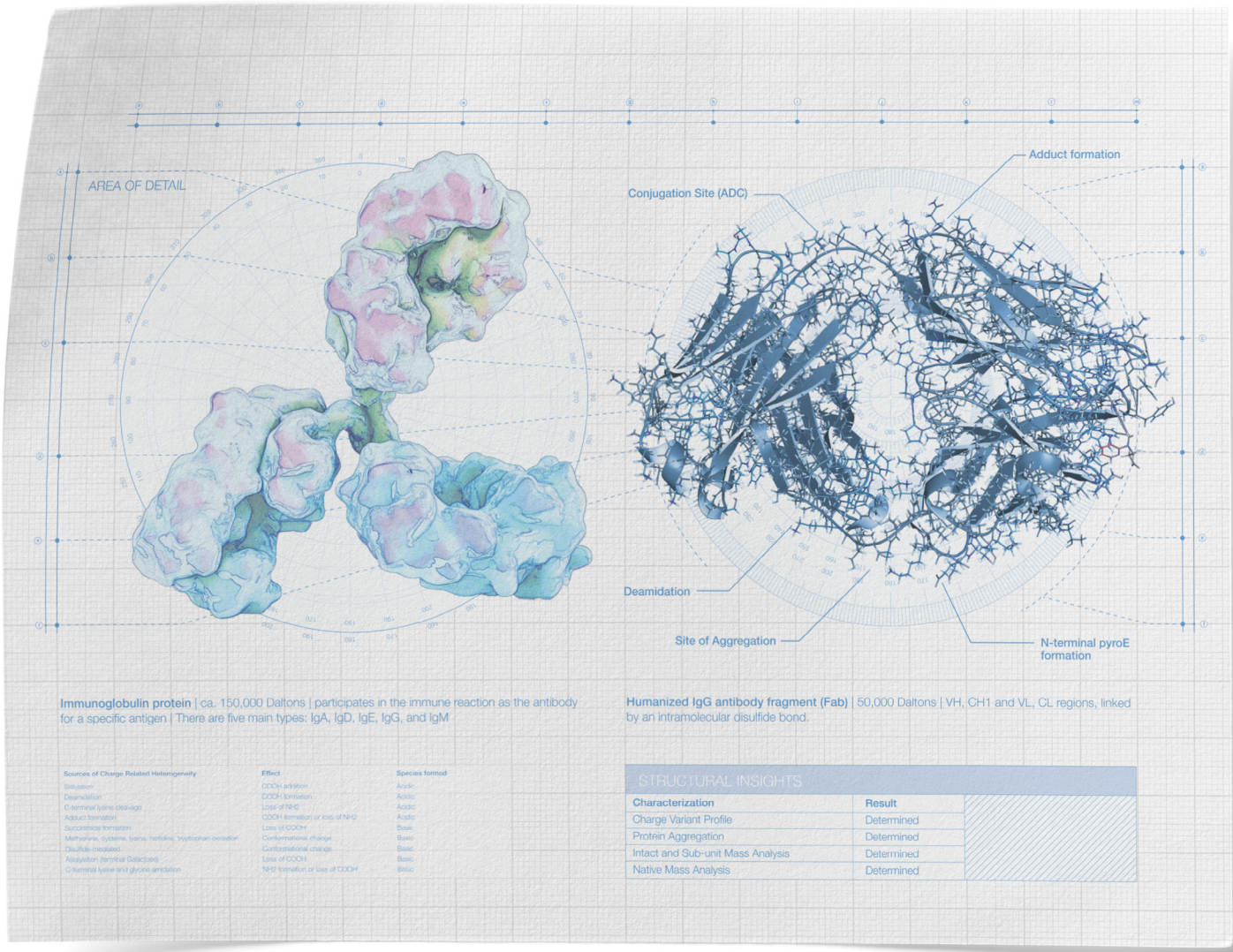

Figure 3. Protein characterization.

Find out more at **thermofisher.com/nativems**

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