A new top-down mass spectrometry method for the rapid analysis of biopharmaceuticals

Working with Covance, Perdita Barran and colleagues from the University of Manchester used BioProNET Proof of Concept funding to develop a rapid and cost-effective method to characterise biologic drugs.

The challenge: Biologic drugs have distinct molecular attributes — such as structural features and post translational modifications (PTM) — that can change their physicochemical and pharmacological properties. Current analytical methods to measure these attributes require separate analysis of the protein and glycan components. And although methods exist for the rapid sequencing of proteins, characterisation methods that involve minimum sample preparation are need.

Project aim: The project aimed to characterize biologics directly from crude cell lysates using top-down mass spectrometry analysis (where intact proteins are introduced into the mass spectrometer). The purpose is to obtain the maximum information on the biological product in the minimum time, with the lowest amount of sample preparation.

Key methods: Three types of mass spectrometry methods were performed on intact biologics — nanobodies and monoclonal antibodies — in crude cell lysates:

- IM-MS ion mobility mass spectrometry
- UVPD-IM-MS – where ion are sequenced with a UV laser prior to mass and mobility analysis
- HDX-MS – where the solution conformation is assessed by hydrogen deuterium exchange coupled with MS

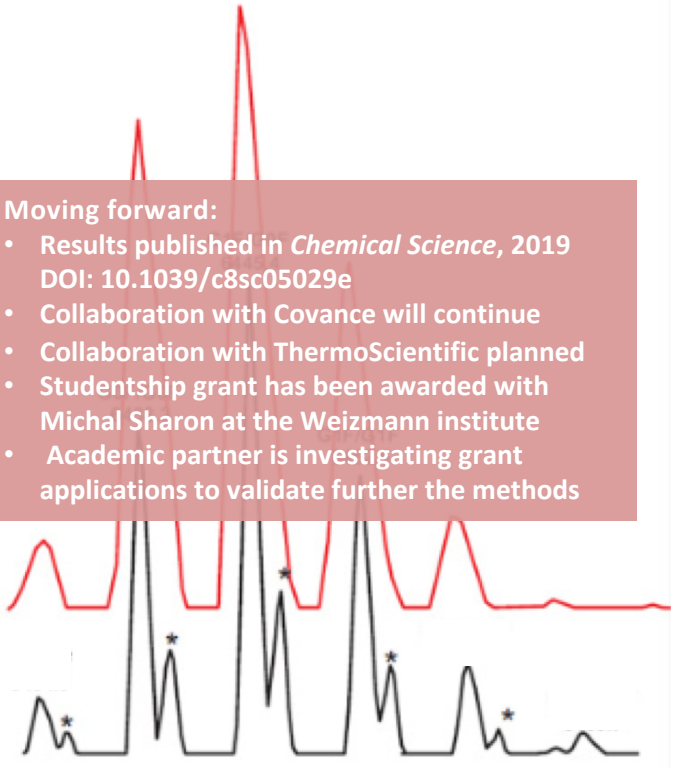
These techniques enabled the measurement of the mass and stoichiometry of the proteins, as well as conformational information and the effects of PTMs and an estimation of glycan content.

"The main outcome is the application of a fast and facile protocol for sample preparation for MS analysis with minimal sample handling that could be easily applied in industrial setting."

Key outcomes: Nanobody cell lysates produced remarkably clean native mass spectra without any further purification. This data provided information on sample heterogeneity, PTMs and aggregation. Importantly, additional purification steps did not uncover any additional information.

Tandem MS experiments on the reduced mAbs allowed accurate prediction of the position disulfide bonds. Moreover, the glycan sequence could be confirmed after the deglycosylation reaction from the same solution without any further sample preparation steps.

The mass spectrometry methods were also used to measure structure variations in the three lots of Herceptin. Despite equivalence at the intact protein level, each lot of Herceptin produced a distinctive signature in the 3 mass spectrometry approaches. This showed that native mass spectrometry can be used to distinguish between lots of an active biopharmaceutical.



Moving forward:

- **Results published in *Chemical Science*, 2019 DOI: 10.1039/c8sc05029e**
- **Collaboration with Covance will continue**
- **Collaboration with ThermoScientific planned**
- **Studentship grant has been awarded with Michal Sharon at the Weizmann institute**
- **Academic partner is investigating grant applications to validate further the methods**