PROTEOMICS

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(see page 52)

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Proteins constitute 50% of the cell (dry) mass and mass spectrometry (MS)-based proteomics is key to unravelling the identity and function of each protein. The proteomics facility is working with cutting-edge mass spectrometry proteomic technologies and innovative platforms for sample preparation and data analysis to answer fundamental questions of cancer biology, thus contributing to the progress of cancer research.

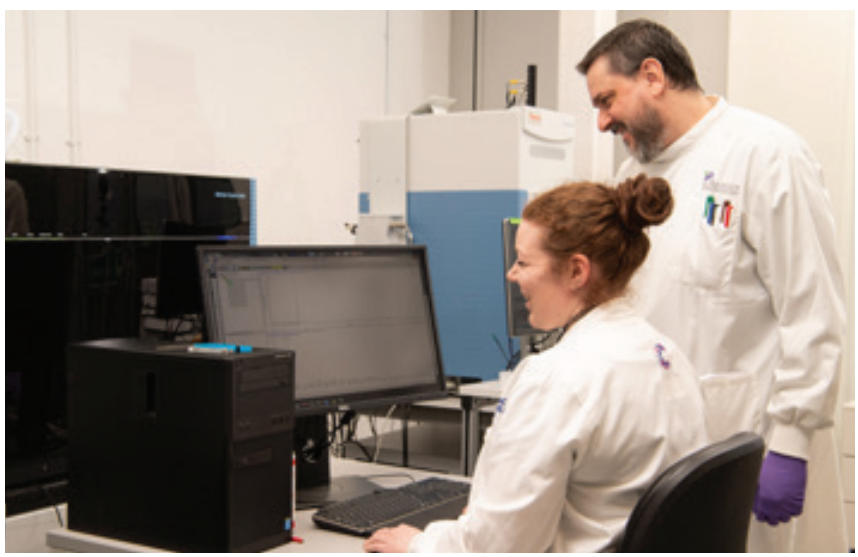
The proteomics team has an outstanding expertise in high-resolution, Orbitrap-based mass spectrometry (MS) proteomics, accurate quantification approaches and MS data analysis. We work in collaboration with research groups at the Institute and outside, and we actively develop MS-based proteomic platforms to address a variety of questions to help scientists better understand the mechanisms that regulate various aspects of cancer.

To achieve this, we are well equipped with three nano-liquid chromatography (nLC)–MS systems. This year we have installed the newest-generation Orbitrap instrument, Fusion-Lumos; we also have a Q-Exactive HF and an Orbitrap Elite. All our instruments are coupled online to EASY-nLC systems, and high-resolution chromatography is achieved by packing our nano-columns in house.

We house a number of dedicated pieces of software, of which MaxQuant is the most used, for highly accurate label-free or label-based quantitative analysis. Moreover, we use Skyline for the analysis of RM data. Finally, we use Perseus and Scaffold for data analysis and dissemination.

This year we have recruited Kelly Hodge as a new senior scientific officer, who is expert in sample preparation for proteomic analysis and MS data analysis. We have also recruited our first bioinformatician, Greg Koulouras, who is expert in web and Android programming and in working with big data and sensitive personal information. With Greg, we are currently working at an ambitious project of developing a cancer-centric database for the navigation through and analysis of MS–proteomic data.

During 2018, we have worked with many of the groups at the Institute and significantly contributed to the success of their research. Following the development of our novel stable isotope-based method, SICyLIA, for the global measurement of cysteine oxidation we are now further developing the approach to enable dynamic measurement of cysteine oxidation and to achieve higher depth of our analyses in a shorter time.



We are continuously striving to develop methods to answer more complex biological questions using proteomics and improve the methods currently in place to enrich the quality of the data that the facility can provide. We have now set up tandem mass tag (TMT)–based labelling approaches to multiplex up to ten samples in one. Moreover, we have been working to improve the depth of the proteomes and sub-proteomes achieved starting with small amounts of sample, e.g. FACS-sorted immune cells, using high-pH reverse-phase LC fractionation. This enables us to perform improved global proteomic, sub-proteomic and post-translational modification analyses of primary cells, circulating blood cells and 3D organotypic cell cultures.

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