

PROTEOMICS



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Proteins constitute half of the cell's (dry) mass and are key functional units that actively contribute to tumour initiation, progression and metastatic spread. Proteins are also used as blood markers to determine the wellness status of an individual. Mass spectrometry (MS)-based proteomics is fundamental to unravel the identity and function of each protein in the cell and body fluids. The Proteomics facility is working with cutting-edge MS 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

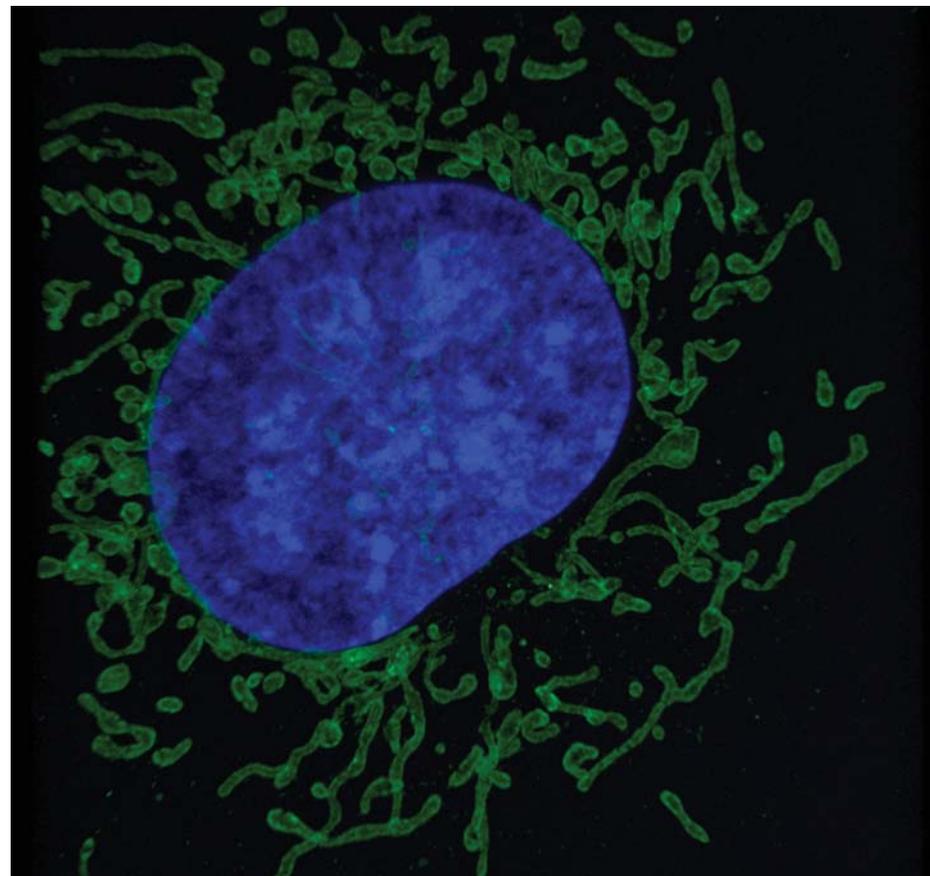
The Proteomics team has an outstanding expertise in high-resolution, Orbitrap-based mass spectrometry (MS) proteomics, accurate quantification approaches and MS data analysis. We work in collaboration with research groups within and outside of the Institute, and we actively develop MS-based proteomic platforms to address a variety of questions to help scientists to increase their understanding of the mechanisms that regulate various aspects of cancer. To achieve this, we are equipped with three nano liquid chromatography (nLC)-MS systems, including an Orbitrap Fusion-Lumos. 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 software packages, of which MaxQuant is most frequently used for highly accurate label-free or label-based quantitative analysis of data acquired in data-dependent acquisition mode and Spectronaut® for data acquired in data-independent acquisition mode. Moreover, we use Skyline for the analysis of PRM data. Finally, we use Perseus for data analysis and dissemination.

We have a competitive portfolio of techniques available, which span from single protein to

sub-proteomes and global proteome analyses. We have strong expertise in quantitative analysis of secretomes (extracellular matrix, extracellular vesicles and conditioned media) and protein translation, including in approaches that allow us to study protein translation dynamics by tracing ¹³C-labelled metabolites and amino acids into newly synthesised proteins (Schmidt *et al.*, 2023, *Nucleic Acids Res*; Kay *et al.*, 2022, *Nat Metab*). We are also experts in posttranslational modifications, particularly cysteine oxidation dynamics, for which we have developed SiCyLIA, a method that enables us to quantify cysteine oxidation levels at global scale with no enrichment steps required (van der Reest, Lilla *et al.*, 2018 *Nat Commun*) and that has been fundamental to answer different biological questions (Cao *et al.*, 2020; *J Cell Sci Part et al.*, 2018, *Cancer Discov*; Hernandez-Fernaudo, Ruengeler *et al.*, 2017, *Nat Commun*).

During 2024, we have worked with many of the groups at the Institute and significantly contributed to the success of their research (see publications). We are continuously striving to develop new methods to answer more complex biological questions using proteomics and to improve the methods currently in place enriching the quality of the data that the facility can provide.



Expansion Microscopy of SVCE cells stained for Mitochondria and Nucleus - Nikki R. Paul and Rosalie Heilig