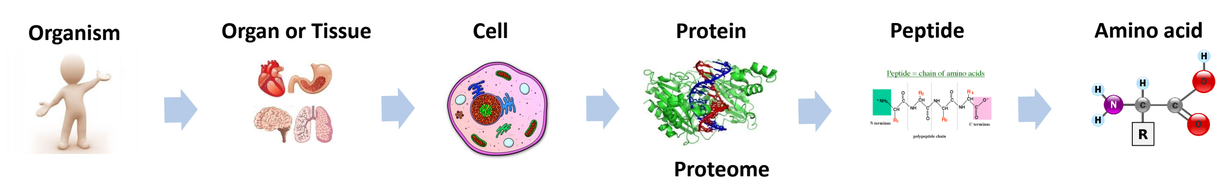
Proteomics step by step

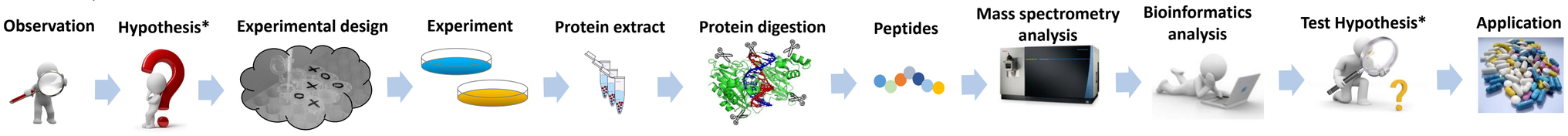
Proteomics

An **Organism** is typically an individual life form composed of interdependent parts (organs). The **organs** have specific functions and they are composed by cells. A **cell** is the smallest structural and functional unit of an organ and is microscopic. **Proteins** do most of the work in cells and are required for the structure, function, and regulation. Proteins are made up of hundreds or thousands of smaller units called **amino acids**, which are attached to one another in long chains. The **proteome** is the entire complement of proteins that is or can be expressed by a cell, an organ or an organism and the study of proteomes at each of these levels is called **Proteomics.**



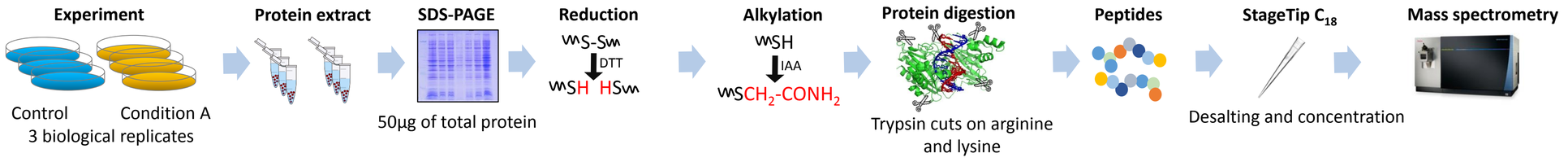
Strategy

The scientific method starts with a problem/question identification and study of relevant previous data. Then, a hypothesis is formulated from these observations and is empirically tested by a specific experiment. At Warwick staff in the Proteomics Research Technology Platform are experts **in mass spectrometry based proteomics** which investigate the proteomes/proteins and their functions. We are here to help you with proteomic experiments.



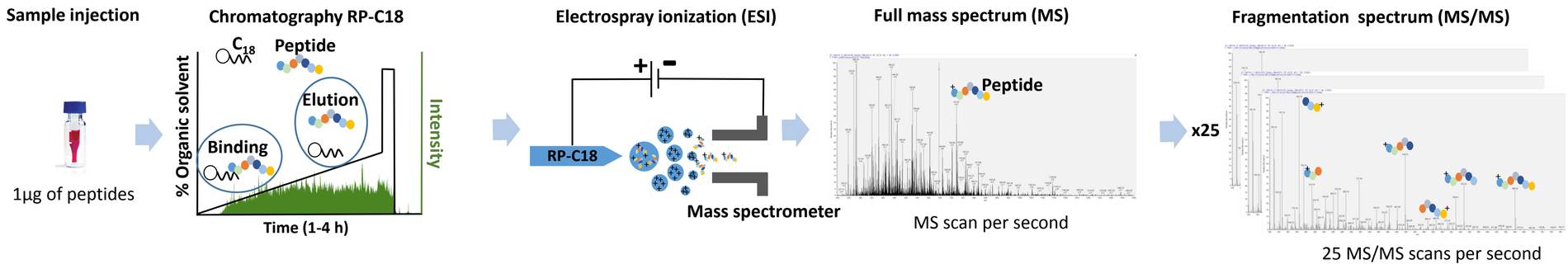
Workflow

A standard **proteomics experiment** compares the protein levels of two or more biological conditions (e.g. Healthy vs Disease). The protein extracts are fractionated to reduce their complexity (often using protein electrophoresis, SDS-PAGE), before in gel digestion to create smaller fragments of proteins called **peptides**. These peptides enable protein identification and quantification by mass spectrometry. Other types of experiments are possible, so please discuss your ideas with us **before** extracting proteins!



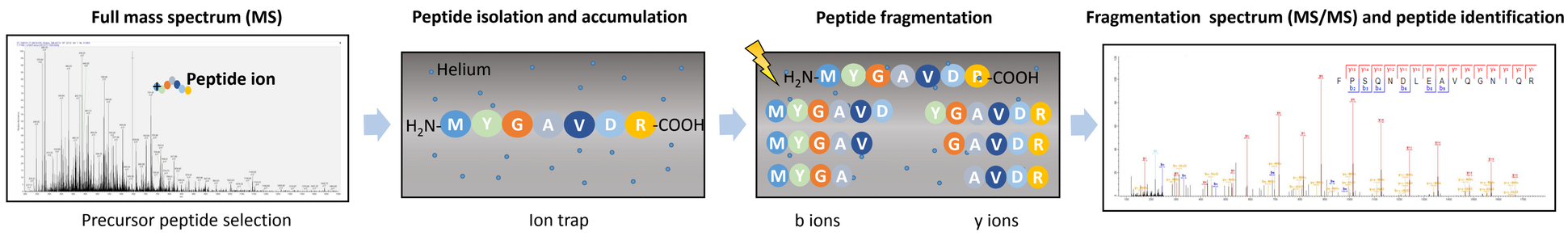
Technology

Mass spectrometric analysis usually starts with chromatography of the peptides by **Reverse Phase C_{18} (RP-C_{18})** coupled with a electrospray ionization source. RP-C_{18} reduces the complexity of the peptide mixture by gradually eluting them off the column using a hydrophobic stationary phase composed by octadecyl carbon chain (C18)-bonded silica. Hydrophobic peptides bind to the column in 0.1% formic acid aqueous solvent and eluted with increasing concentration in a linear gradient of acetonitrile (organic solvent). Eluted peptides are desolvated and ionised by **Electrospray ionization (ESI)**. The positively charged peptides (now called ions) fly down a voltage gradient into the mass spectrometer to strike a detector for analysis. The mass of the peptide and their fragments is calculated from the flight path.



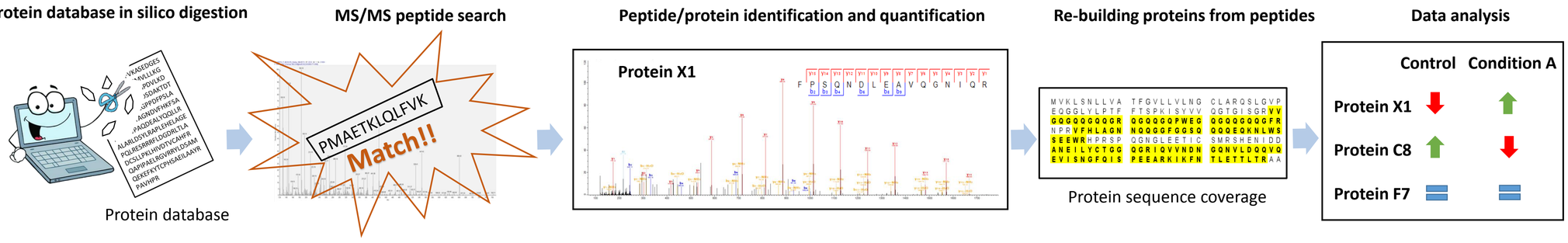
The trick!

Data dependent analysis (DDA) first selects peptide precursors from a full mass spectrum scan (MS) and then acquires MS/MS scans centered on these chosen masses. The cycle of selection and fragmentation is then repeated. The selected, individual peptides are accumulated in the ion trap and fragmented upon an energy pulse in the presence of an inert gas (collision gas). All fragments are measured in a new mass spectrum (MS/MS). Finally, search engine software uses known protein sequences to match the peptide fragmentation patterns (MS/MS).



Results

Protein and peptide **identification software** uses known protein sequences to match the calculated in silico protein digestion and peptide fragmentation with the real peptide fragmentation acquired in the mass spectrometer. Each identified mass in the MS is assigned to a peptide and each mass in the MS/MS is assigned to a peptide fragment. All peptides that belong to the same protein will contribute to its identification and relative quantification. Complex algorithms combine the peptide intensities from the same protein and calculate the protein relative abundance. Finally, data analysis involves **relative quantitative comparison** and selection of **regulated proteins** as a candidates for further investigations.



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