Pharmacologically, pathogens target membrane components. Our cells are surrounded by membranes, which act as gatekeepers, allowing certain molecules to enter and exit while maintaining the integrity of the cell. Membranes are composed largely of lipids and proteins, and because of their important role in protecting our cellular contents, many pathogens have evolved to attack or even hijack these components. In fact, more than half of all new pharmaceutical drugs target membrane proteins.

Pore-forming toxins (PFTs) are unique membrane proteins that are produced by pathogens in all kingdoms of life. They are unique membrane proteins in that they produce an aqueous-soluble monomer that transitioning into membrane-inserted oligomeric pores. This action can activate cell death, membrane repair, and immune responses in the host cells. Despite their importance in a variety of inter- cellular biological processes, PFTs and other membrane proteins are quite challenging to study using traditional methods as they aggregate into water-soluble monomers that transition into membrane-inserted oligomeric pores. This action can activate cell death, membrane repair, and immune responses in the host cells.

Native mass spectrometry allows us to study protein complexes in their native-like conformation. In native electrospray ionization mass spectrometry (native ESI-MS) experiments, the protein sample is first gently sprayed by applying it from an aqueous buffer directly into the mass spectrometer. At the end of the instrument is a detector which outputs a value of m/z, or the mass-to-charge ratio of the ion. Our Waters Synapt G2-Si mass spectrometer also has the capability to select for ions of certain m/z ranges, dissociate or break apart large complexes, and to obtain protein size and shape measurements.

**Methods**

**Native mass spectrometry**

- **Fractionation and isolation of proteins:** The first step in native ESI-MS analysis is to fractionate and isolate the protein of interest. This is typically done using chromatographic methods, such as reversed-phase HPLC or size-exclusion chromatography.

- **Mass spectrometry:** The isolated protein is then analyzed using a mass spectrometer. Native ESI-MS is a technique that allows for the intact analysis of protein complexes. The mass spectrometer measures the mass-to-charge ratio (m/z) of ions produced by the sample, allowing for the determination of the molecular masses of protein complexes.

- **Data analysis:** The mass spectrometry data is then analyzed to identify and quantify protein complexes. This is often done using software tools that can deconvolute complex mixtures into their constituent proteins.

- **Validation of results:** The results of the native ESI-MS analysis are then validated using other experimental techniques, such as X-ray crystallography or cryoelectron microscopy.

- **Results:** The results of the native ESI-MS analysis can provide valuable insights into the structure and function of protein complexes in their native-like conformation. They can also help to identify potential drug targets and to understand the mechanisms of action of therapeutic agents.

**Electrospay MS**

- **Electrospray process:** The protein sample is first dissolved in an aqueous buffer and then electrospayed directly into the mass spectrometer. This process allows for the formation of ions that correspond to the native-like conformation of the protein complex.

- **Mass spectrum:** The mass spectrum obtained from the electrospay MS experiment can be used to determine the molecular mass and stoichiometry of the protein complex.

- **Analysis of results:** The results of the electrospay MS analysis can be used to validate the findings from the native ESI-MS analysis and to further elucidate the structure and function of the protein complex.

**Conclusion**

Native ESI-MS and electrospay MS are powerful techniques for the intact analysis of protein complexes in their native-like conformation. These techniques have been used to study a wide range of biological systems, including membrane proteins, protein complexes, and protein-ligand interactions. The results of these experiments have provided valuable insights into the structure and function of these systems and have helped to identify potential drug targets and to understand the mechanisms of action of therapeutic agents.