

IMPROVING TOP-DOWN PROTEOMICS: FIRST STEPS

C.Fuentes⁽¹⁾, R.González⁽¹⁾, C.Gómez⁽¹⁾, S.Ogueta⁽¹⁾

⁽¹⁾Proteomics Core. SCAI. University of Córdoba. Campus Rabanales. 14071 Córdoba. Spain

Proteomic analysis use to be addressed by bottom-up approaches. This is the easiest way to identify and characterize single proteins or a mixture of them. However, it is also a time consuming process that performs several steps that are not still well controlled, i.e. digestion efficiency. Theoretically, most of those problems could be diminished by applying top-down based approaches, where entire proteins are analyzed without any major treatment. We are now starting to improve top-down methodology to apply it to protein identification as bottom-up approaches. In this sense, to date, top-down analysis is performed by direct injection of purified proteins on a FT mass spectrometer. MS/MS spectra are obtained by CID fragmentation of one of the multicharged ions. In our laboratory, first, we have tuned those conditions on a LTQ-Orbitrap mass spectrometer by infusing a mixture of α and β hemoglobin. Using several hemoglobin variants from different patients, we have achieved a rapid method to highlight possible hemoglobinopathies in newborns and adults by direct infusion of periferical blood on the Orbitrap mass spectrometer. Then, we intended to perform a real time top-down analysis of a protein mixture by LC-MS. The main problem with this approach was the generation of good quality MS/MS spectra during the chromatographic run. Proteins below 20kDa in molecular mass generate MS/MS spectra of acceptable quality when separated by LC, but higher molecular mass proteins not. In order to solve this problem, we have tested the possibility of CID fragmentation of protein fragment that were obtained after in source fragmentation. Although better results were obtained, more studies are needed in this area.