Virtual 2D gel/MS combines proteome-scale separation with MALDI mass spectrometry. To demonstrate this capability, we employed the method to analyze different cultures of *Escherichia coli* strain K12, some of which had been exposed to various forms of stress. This included low concentrations of the antibiotic actinonin and reduced temperatures. Employing a recently developed overnight matrix application method, high quality, reproducible MALDI spectra were acquired. A custom, in-house, MATLAB program was used to generate the virtual 2D gel images. Total time for MALDI acquisition and virtual gel generation total less than one hour for gels of typical length, 18 cm. These gel images were processed through both visual analysis and computational techniques to characterize the differences in the E. coli cell lysate. In the cold shock experiments, the appearance of an abundance of cold shock protein along with a lack of other protein was observed in the experimental lysate.

Collisional induced unfolding (CIU) offers a unique way to probe protein structure and their stability by slowly exciting the complex and monitoring changes to its collisional cross section. Using the CIU technique, we analyze how ligand affects the stability and structure of α-synuclein. Investigation by CIU shows that manganese (II) provides moderate stabilization for the lower charge states when bound to α-synuclein (relative to no metal binding present). A similar result is seen when studying cobalt (II) bound to α-synuclein. It appears that CLR01, a lysine specific molecular tweezer that binds noncovalently and inhibits the aggregation of α-synuclein aggregation, also stabilizes the structure of the protein, as probed by CIU. This is interesting because the heavy metals, which promote aggregation, have the same effect on protein stability. Comparing the CIU profiles measured for α-synuclein with different ligands help to link the role of protein structure and aggregation processes important in neurodegenerative diseases.