Can we correlate ion mobility mass spectrometry data with native solution structures? A crosslinking approach.

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It is now well-established that the structural characterization of biomolecules and biomolecular complexes is of prime importance in the understanding of biological processes at the molecular level. Methods such as X-ray crystallography, NMR spectroscopy and electron microscopy are widespread tools that are used to infer structural data, but may sometimes be challenging and laborious to set up. In this context, the development of the so-called soft ionization techniques, namely electrospray ionization (ESI), opens new perspectives for the analysis of large intact molecular ions by mass spectrometry (MS). In addition, the advent of ion mobility (IM) separation techniques interfaced with MS now enables the study of the global size and shape of specific mass-to-charge resolved ions. From this angle, previous (IM-)MS experiments emphasized that the solution phase structures may be, at least partially, preserved in the gas phase under soft ionization conditions. However, it is known that several factors have an influence on the conformation of biomolecular ions in the gas phase and should therefore be carefully considered. These are either dependent on the ion nature, *i.e.* the Coulomb repulsions between charged sites, or conditioned by experimental parameters such as the various accelerating voltages required to propel the ions along the instrument path. Consequently, some fundamental questions remain elusive: "*What are we probing in the gas phase?" - "Can we correlate ion mobility mass spectrometry data to native solution structures?"*

To bring innovative answers to these longtime debated questions, we here propose an early stage study targeting constrained "native-like" conformations in the gas phase. To this end, we capitalize on a crosslinking approach to establish chemical links between lysine residues located in close spatial proximity within the protein. The so-created covalent network is here used as a scaffold to prevent the collapsing and unfolding of biomolecular ions once desolvated in the gas phase. These "frozen" native solution conformations are then interrogated using ion mobility as implemented on the commercial SYNAPT G2 HDMS spectrometer.

Practically, we used cytochrome *c* as a model system that we crosslinked with BS^3 linkers. Using ion mobility, we monitored the evolution of the collision cross section (CCS) quantity, a rotationally averaged 2D projection of the ion conformation, for each charge state as function of the number of covalent intramolecular linkers. These data were readily compared with those obtained in similar conditions for the non-crosslinked cytochrome *c* as well as with a benchmark corresponding to the native solution conformations as resolved by NMR spectroscopy. Our results highlight that the crosslinked cytochrome *c* adopts more compact conformations in the gas phase, closer to values monitored for the native solution conformation, compared to its non-crosslinked homologue. In addition, we found that a critical number of intramolecular linkers were required to prevent structural unfolding from Coulomb repulsions.

Altogether, this preliminary work opens new perspectives for the use of crosslinking methodology in the native MS field. This may certainly contribute to facilitate the understanding and the interpretation of native-like conformations isolated in the gas phase.