Contribution ID: **43** Type: **Oral Presentation**

X-ray spectroscopy meets native mass spectrometry: probing gas-phase protein complexes

Wednesday, August 27, 2025 11:20 AM (5 minutes)

X-ray activation and dissociation of proteins and their non-covalent assemblies may elucidate structural and functional details complementary to established top-down mass-spectrometry techniques. This is attributed to the rapid, site-specific ionization of atoms within biomolecules. Our research group conducted proof-of-concept experiments exploring X-ray activation of samples with masses ranging from small 17 kDa monomeric proteins to large 800 kDa non-covalent protein complexes at synchrotron (PETRA III) and free-electron laser (FLASH2) facilities. A quadrupole time-of-flight mass spectrometer, adapted for high-mass analysis, was further modified to enable photon-ion interactions. Native proteins and their complexes were introduced into the gas phase via nano-electrospray ionization and exposed to either extreme ultraviolet (FLASH2) or soft X-ray (PETRA III) radiation, in either their native folded state or following gas-phase collision-induced activation. The resulting effects—fragmentation, dissociation, or enhanced ionization—varied depending on biomolecule size and activation method. We also explored the integration of ion mobility to enhance structural separation prior to X-ray probing. Within the rapidly evolving domain of X-ray technologies, the activation of large proteins and their complexes via X-rays holds significant potential for advancing top-down analysis and structural biology research.

User consent

yes

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Session Classification: Lightning Talks