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Heparin-like disaccharides: effects of metallic complexation in fragmentation pathways as characterized by MS/MS and UVPD experiments

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Motivations :

Why Heparin disaccharides?

Heparin (HP) glycosaminoglycan (GAGs)¹, an anticoagulant drug, is recognized to be a biologically important polysaccharide. It has been **implicated in many biological processes** such as blood coagulation, cell-cell and cell-matrix interaction inflammatory processes, cell growth, lipid transport and metabolism.

Why is it important to study the interaction between HP and metal cations?

The effect of metal ions on protein-carbohydrate complexes is largely **unknown**. Heparin-biomolecule interaction can be influenced by the binding of metal ions to these complexes². For example, it has been reported that physiological Ca^{2+} **induces conformational changes** in heparin that are necessary for the interaction between the anticoagulant Heparin and Annexin V, a protein proposed to play an important role in the inhibition of blood coagulation³. Then so, it is a **Calcium-depended interaction**.

What is our strategy?

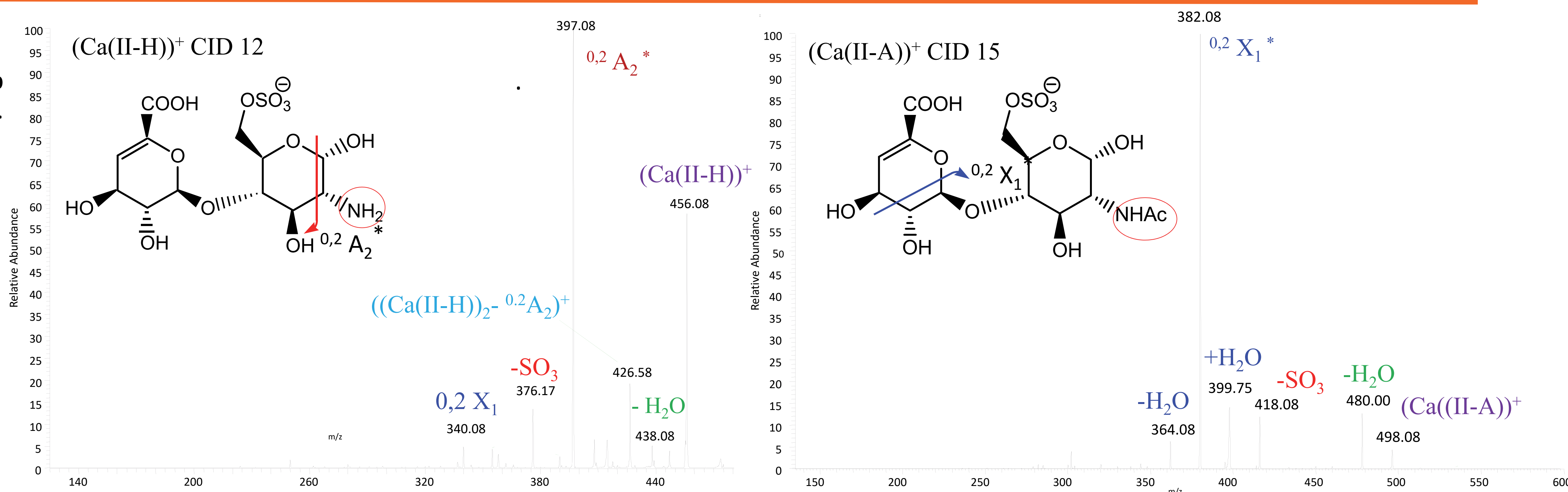
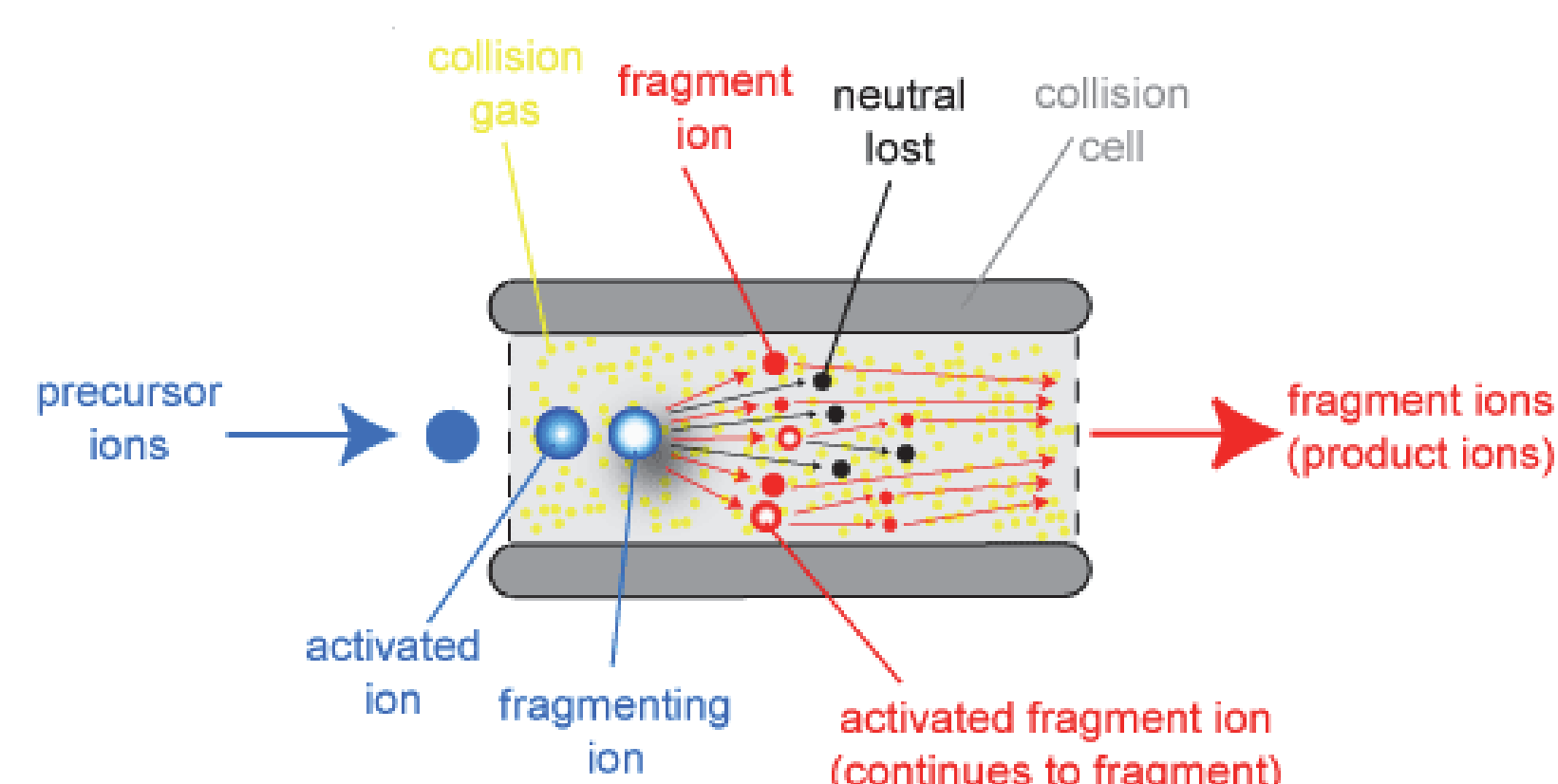
Experimentally, our aim was to study $(\text{Ca}(\text{II-H}))^+$ and $(\text{Ca}(\text{II-A}))^+$ complexes by tandem **ESI/MS**. Once generated in the gas phase, ions then undergo a fragmentation process by Collision Induced Dissociation (CID). **Without** metal there is **no difference** in the MS/MS spectra. Only $^{0,2}\text{A}_2$ fragmentation is observed. Ca^{2+} cation induces different conformational changes in both isomers, resulting in **completely different** fragmentation pathway. Interaction between Acetyl/ Ca^{2+} must be important in the dissociation process. Theoretically our aim is to explain this Metal-HP interaction by **DFT** calculations and **delineate** mechanisms of dissociation accounting for the experimental data.

Why UVPD photodissociation?

Ultraviolet photodissociation (UVPD) of positive and negative precursor ions appears to be important activation method for providing **complementary** structural information to CID. UVPD favored additional cross-ring cleavages of A and X type ion series, therefore enabling **easier sulfate group location**.

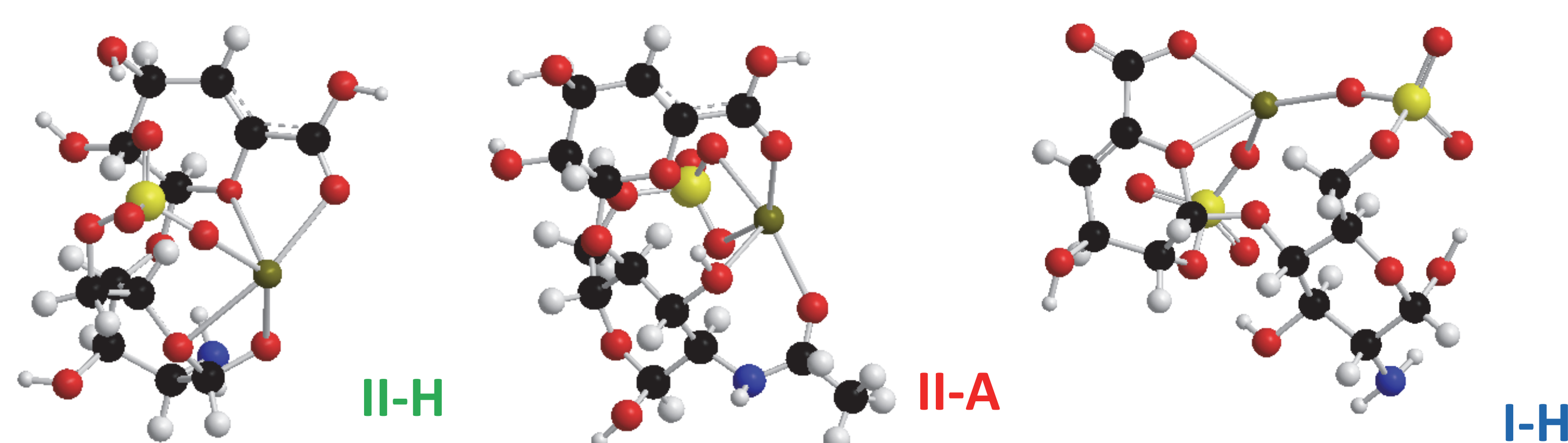
MS/MS results:

Experiments were carried out on a **LIT Orbitrap XL** mass spectrometer coupled to an ESI source. Nitrogen gas was used as collision gas.



Computational results:

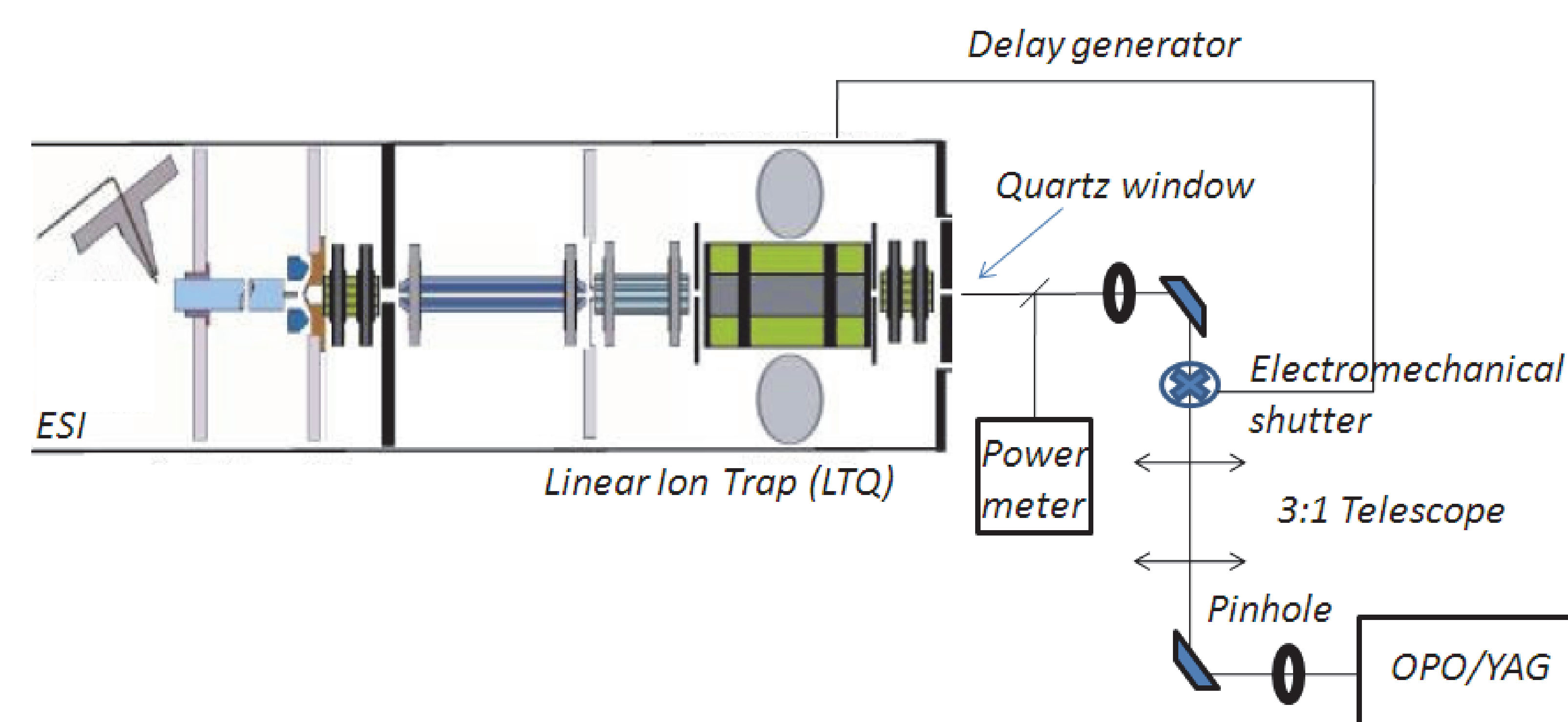
The geometries were optimized using the density functional theory (DFT) with the **B3LYP** hybrid functional and 6-311G** basis set. Refined relative energies were obtained at **6-311++G(3df,2p)** level. **Without Ca^{2+}** all the calculated 50 conformers, for each disaccharide, are very close in energy (**<50 KJ/mol**). Nevertheless, when **$(\text{Ca}(\text{HP}))^+$** is formed it is observed an increase in relative energies between conformers (**>50KJ/mol**).



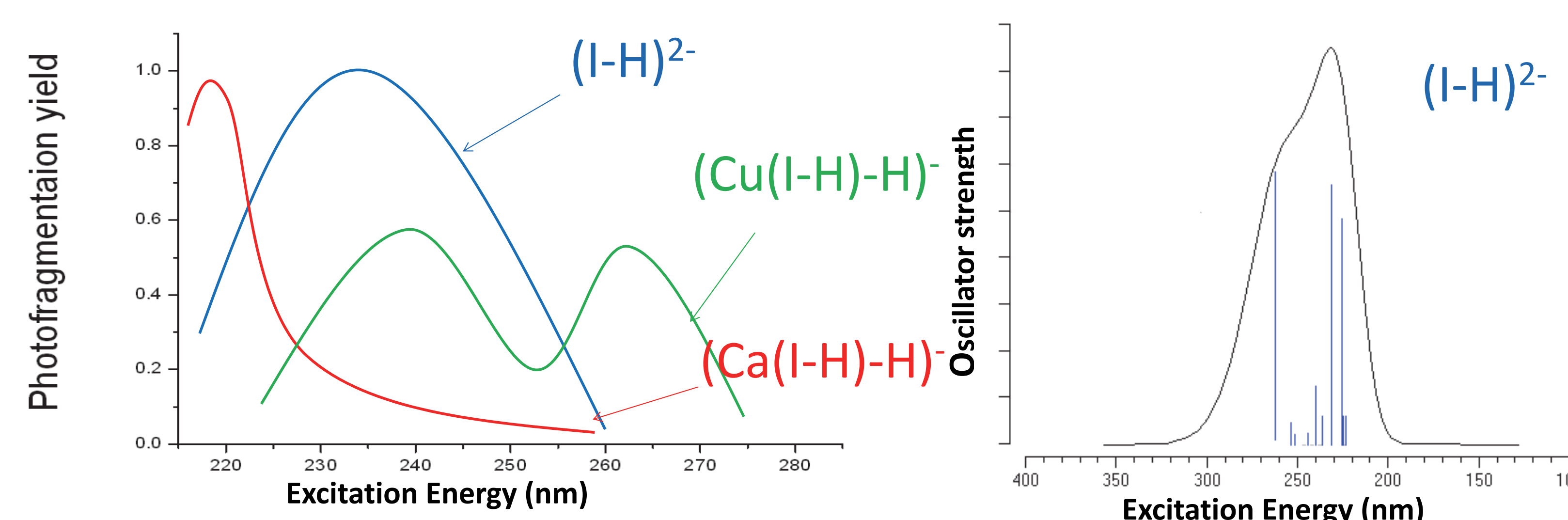
High Binding Energy (BE) values (**~1400 KJ/mol**) are obtained. As deduced from the conformers calculation and the BE values, the metal complex **stabilizes strongly** one structure. It seems reasonable to deduce that both sugars lose partially their possibilities to change structurally. **Biologically**, this consideration could be critical in order to explain the **strong interactions** aforementioned. **Analytically**, when $(\text{Ca}(\text{HP}))^+$ is formed, the molecule loses its flexibility due to the fixation structure effect and therefore it is noticed a decrease in the number of fragments.

MS/UVPD results:

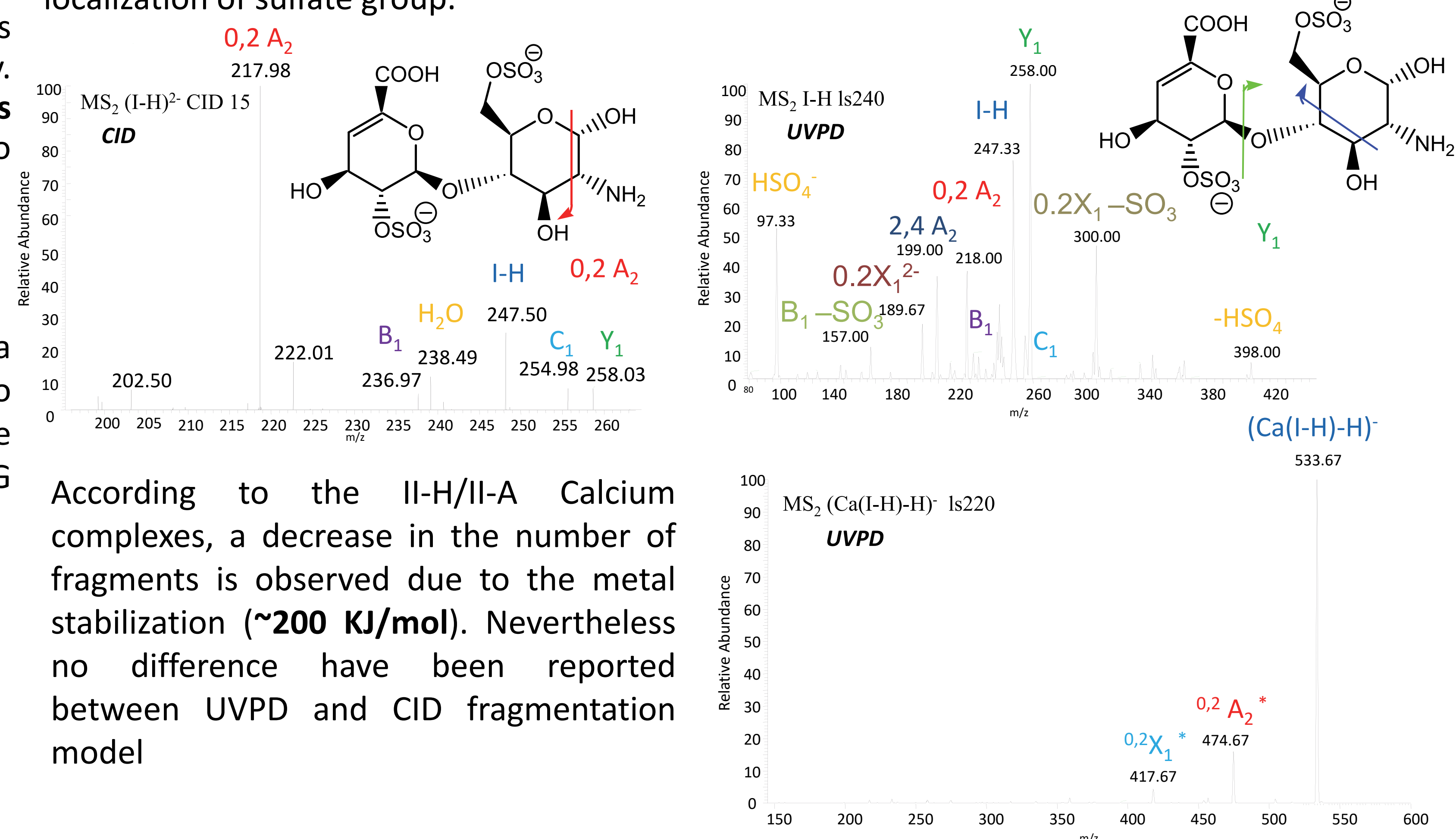
Experimental setup (below) consists of a **LIT-quadrupole linear ion trap** coupled to a visible/UV tunable OPO laser. A quartz window was fitted on the rear of the LTQ chamber to allow the introduction of the laser beam. Once the ions are **isolated** in the trap, they are **irradiated** with the OPO laser (Panther OPO laser pumped by a 355-nm Nd:YAG Surelite; Continuum) and the photofragmentation mass spectra can be recorded.



Optical spectra (Left figure) were obtained recording UVPD spectra as a function of the laser wavelength. Right figure shows the **theoretical UV** spectrum for $(\text{I-H})^{2-}$. It was obtained at B3LYP/6-311++G(2df,2p) level with TD(nstates=20).



CID and UVPD (at 240 nm) spectra for I-H^{2-} were also been recorded. Most of the fragment ions resulting from CID were also observed in UVPD mode. As it was already reported⁴, additional cross-ring fragments in UVPD mode ($^{0,2}\text{A}_2$) provides more information about the localization of sulfate group.



According to the II-H/II-A Calcium complexes, a decrease in the number of fragments is observed due to the metal stabilization (**~200 KJ/mol**). Nevertheless no difference have been reported between UVPD and CID fragmentation model

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