TopSpec - 829157

WP3 - Development and application of H-atom bombardment (HAB) MS/MS techniques

Deliverable: D3.1 - Prototype of the HAB gun installed and tested protocol

Content:

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Introduction

Work package 3 is concerned with the development of a hyperthermal Hydrogen Atom Bombardment (HAB) source for the investigation of gas phase reactions between energetic hydrogen atoms and protein ions stored in the omnitrap platform. Experiments performed so far with a thermal version of the hydrogen atom source have produced exciting results, which include hydrogen attachment to radical fragment ions as well as an unusually high hydrogen atom uptake observed for heme proteins. Nevertheless, the applicability of the thermal version of the HAB source is limited by the low reaction rate of hydrogen atoms. Extending the energy range in these reactions will enhanced the efficiency of the process, improve sequence information in protein analysis, and overall, expand the arsenal of ion activation methods and the versatility of the omnitrap platform for tandem MS experiments. **Figure 1** shows the Gantt chart highlighting the development stages and milestones of the HAB source within the framework of the TopSpec project.

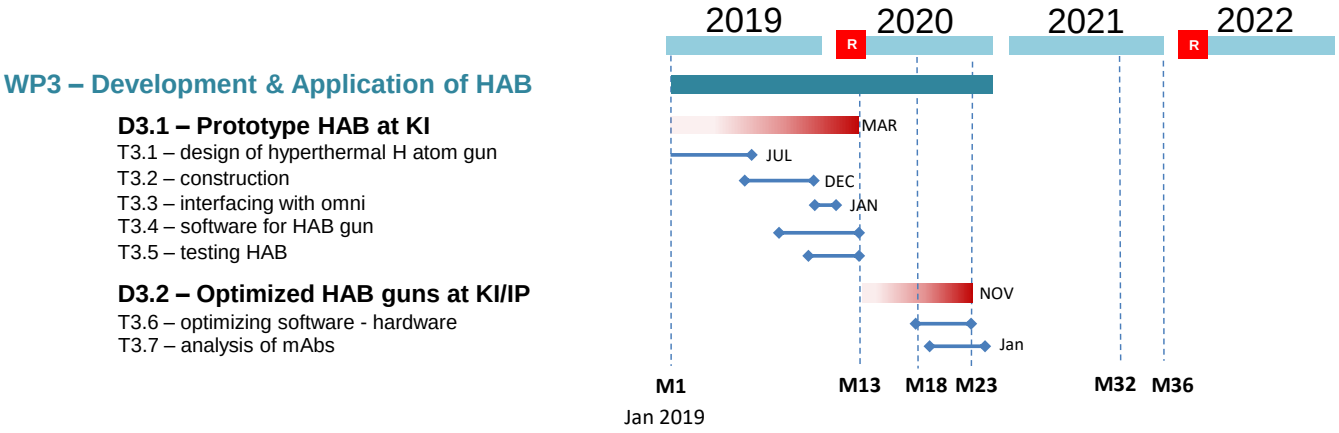

Figure 1. Development stages and milestones for the Hydrogen Atom Bombardment (HAB) source.

As outlined in the Periodic Report, the design, construction and interfacing phases of the HAB source have been completed on time (T3.1-T3.4). A brief summary is provided here for conciseness including additional information describing most recent developments. Preliminary results with the new H-atom source are presented. A patent describing the design features of this new scheme of a free jet plasma ion/atom source and applications in ion trap mass spectrometry is under preparation.

Tasks 3.1 – 3.4

The design of the HAB source is based on a free-jet plasma where molecular hydrogen is injected by a pulse valve through a ceramic flow tube into vacuum. A Dielectric Barrier Discharge (DBD) is established by biasing a conductive element inserted in the flow tube to a high positive or a negative voltage level. Plasma products including electrons, ions and metastable species are released into vacuum in the form a free jet expansion. A series of ion optical elements disposed downstream in the far field region of the free jet are utilized to separate neutrals from ions, collimate and also neutralize fractions of product ion species en-route to the omnitrap. The design has been developed and investigated experimentally in a

separate rig. Details of these efforts were presented in the Period Report. The finalized design of prototype HAB source for connecting to the omnitrap platform is presented in **Figure 2**.

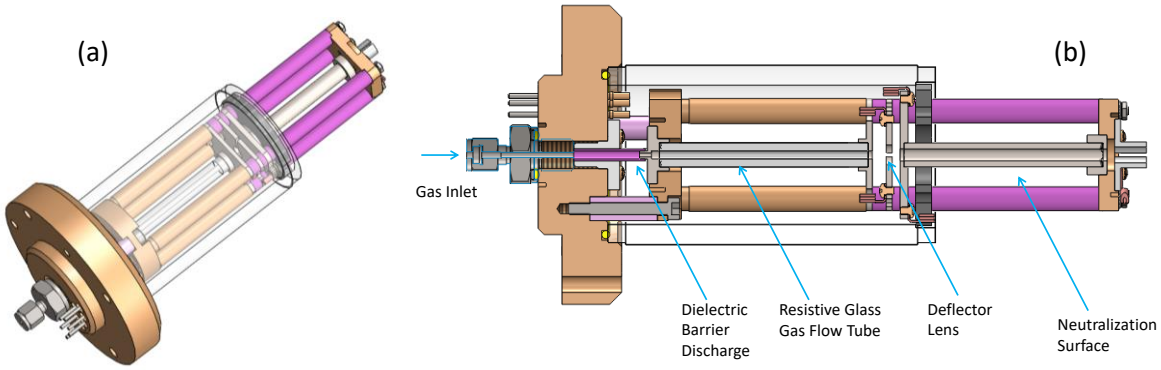

Figure 2. (**a**) 3D model of the HAB source, (**b**) cross section highlighting the different regions of the system where plasma ionization, charge polarity separation and surface neutralization process are taking place.

A few additional characteristics of this new source for activating trapped ions are briefly described here. The strict vacuum requirements of the orbitrap mass analyzer are satisfied by injecting pulses of gas instead of admitting gas continuously that would result in an elevated pressure environment. Pulsed operation of the DBD source is generating transients of ion current of the order of a few tens of ms. It is understood that the pressure gradient established across the flow tube is very strong and thermalization of ions via collisions inside the flow tube appears to be less effective as originally anticipated. The maximum background pressure during the free jet transient does not exceed the 0.1mbar threshold.

The assembled prototype is presented in **Figure 3** (**a**). **Figure 3** (b) shows a cross section of the 3D CAD model highlighting the arrangement of the HAB source relative to segment Q5. In this arrangement the neutralization surface has been removed and reagent ions produced in the free jet plasma are injected directly into the omnitrap. A series of preliminary tests to investigate reactions between hydrogen ions and protein species have been performed and results are presented further below.

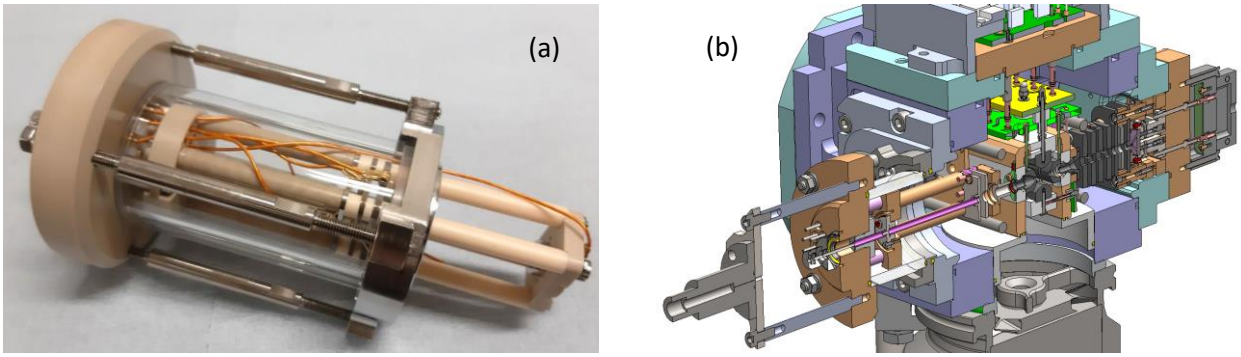

Figure 3. (**a**) HAB source assembled and (**b**) 3D CAD model showing the first configuration of the HAB source coupled to the omnitrap and investigated experimentally. These first tests exclude the neutralization surface – plasma products are focused and injected directly into Q5.

All electronics have been completed and tested successfully. Modifications to the omnitrap control software include 1) additional trigger signal introduced into the instruction sequence list for driving the pulse valve in a synchronized manner, 2) a series of DC potentials and 3) a deflecting voltage pulse to control the characteristics of the ion-neutral beam transmitted into the omnitrap segment Q5. All these new outputs have been tested successfully. **Figure 4** shows the omnitrap user-interface. The additional parameters for driving the HAB source and the instruction sequence list developed for testing interactions between the free jet plasma species and trapped protein ions are highlighted.

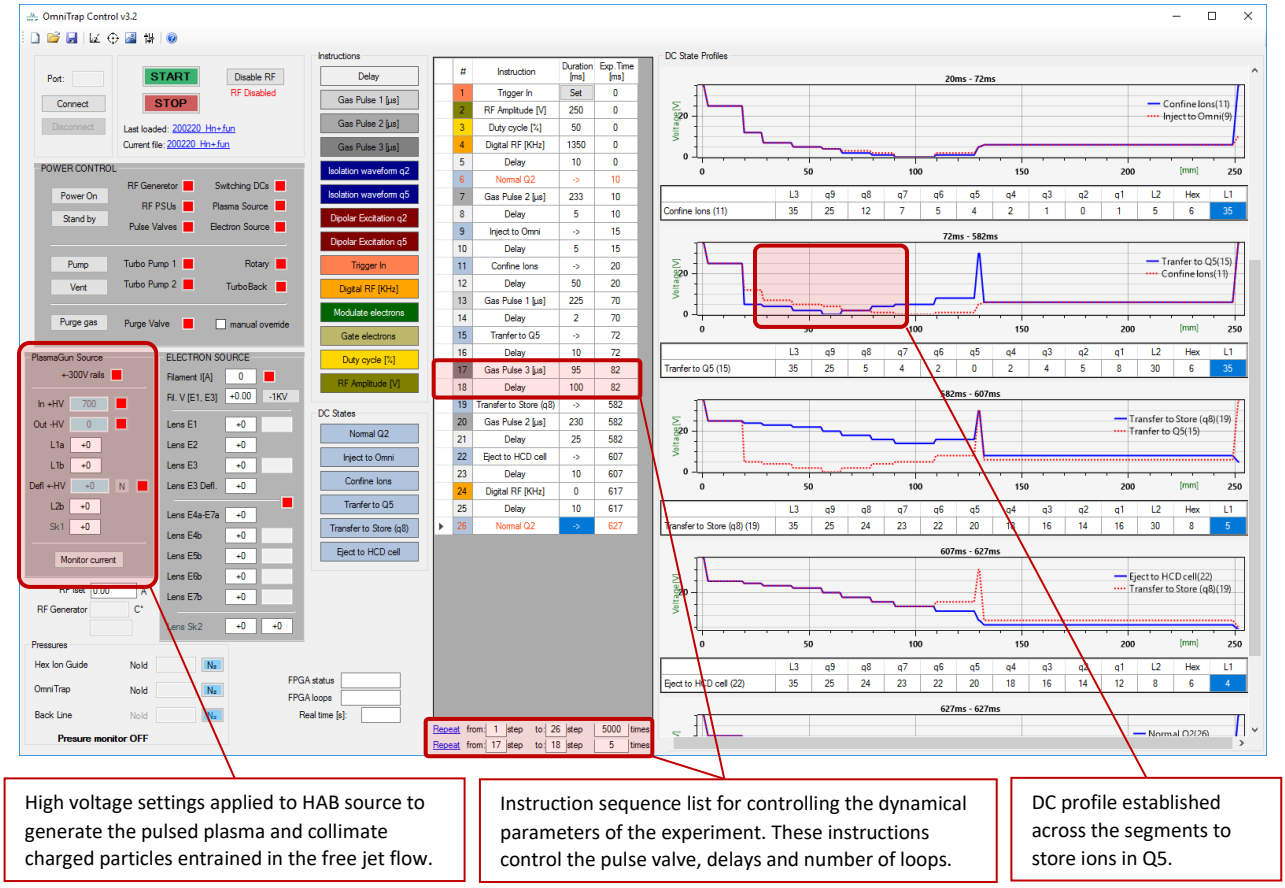

Figure 4. Latest version of the omnitrap UI designed to operate the new HAB source.

Task 3.5

A preliminary round of experiments has been performed successfully. Interactions between multiply protonated myoglobin and product ions produced in the free jet hydrogen plasma are reported. **Figure 5** shows oscilloscope traces recorded on the skimmer-lenses S1 and S2 connected on either side of segment Q5 respectively. For a +1KV potential applied to the DBD electrode, a value of <-1.7KV applied to the exit lens of the flow tube is necessary for the ion current to exceed the 10µA threshold. Increasing the negative voltage applied to the exit lens increases the ion current injected in the direction of Q5 further (positive ion current is measured on S1). The corresponding transients of ion current recorded

on the exit skimmer lens of segment Q5 (positive ion current measured on S2) are also shown and indicate that several microampere of ion current are transmitted through the trapping region for a period of ~1ms (per gas pulse).

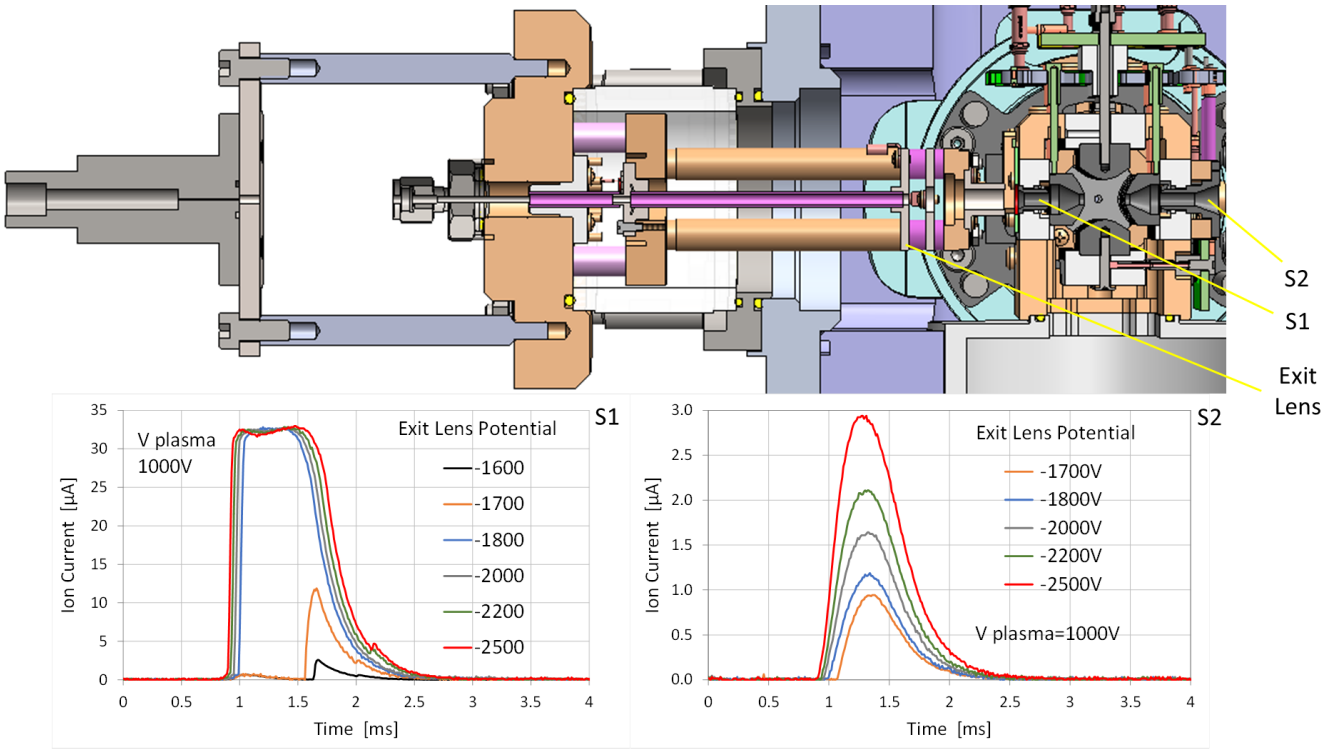

Figure 5. Experimental determination of the pulsed ion current produced in the HAB source and injected through segment Q5. Ion Current transients are measured on skimmer-lens electrodes S1 and S2 at the front and rear of segment Q5 respectively.

The effect of injecting multiple gas pulses through segment Q5 where myoglobin $[M+9H]^{9+}$ ions are isolated and stored is presented in **Figure 6**. Different effects are observed and results are summarized here. A first effect observed in this experiment is multiple ionization of the precursor ions to form hydrogen deficient radical species, in this example $[M+9H]^{10+\bullet}$ and $[M+9H]^{11+\bullet\bullet}$. The reaction can be described as follows:

$$[M+nH]^{n+} + H_n^{+}{}_{(n=1,2,3)} \longrightarrow [M+nH]^{(n+1)+\bullet} + e^{-} + H_n^{+}$$

Another aspect of this ion activation approach is the formation of charged-reduced species, the second most abundant product species observed in reactions between multiply protonated proteins and hydrogen plasma product ions. It is not clear whether the charge reduced species are a result of proton abstraction alone $[M+8H]^{8+}$ or whether the charge-reduced ions are H-atom enriched, $[M+9H]^{8+\bullet}$. Distinguishing between the two different product ions requires a more precise mass calibration

procedure. The formation of H-atom enriched protein ions is an important aspect of the TopSpec project.

In addition to the ionized and charged reduced precursor ions discussed above, a series of fragment ions in both the low and high m/z range are also observed. Fragments ions in the low m/z range are not expected to be sequence informative, however, high m/z fragments are of great interest and particularly suited for exploiting the multiple-stage tandem MS capabilities of the omnitrap platform further. The intensity of the low m/z fragments increases disproportionally as the amount of plasma ion current injected through segment Q5 increases (Exit-lens potential <-2000V).

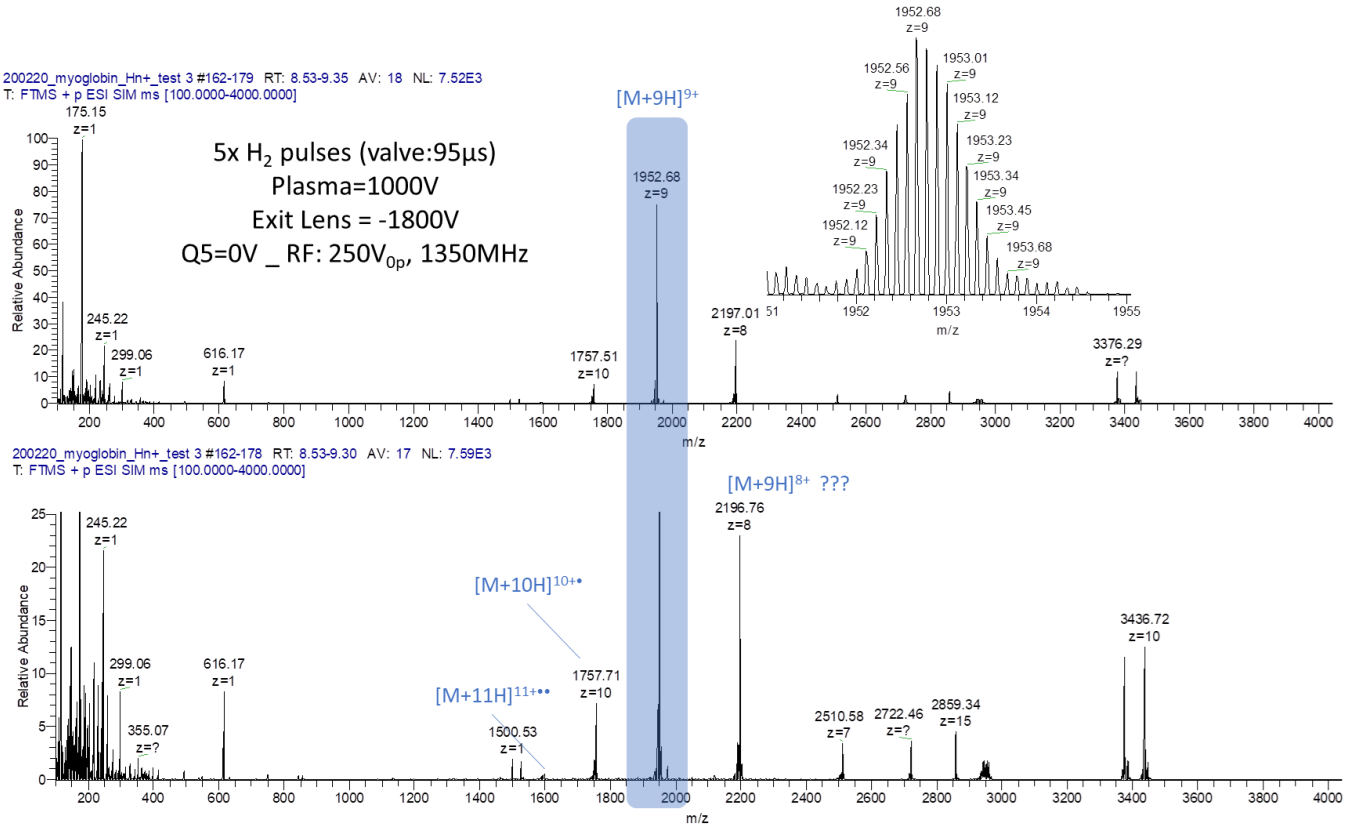

Figure 6. MS/MS spectrum of $[M+9H]^{9+}$ ions of myoglobin irradiated with five consecutive gas pulses from the HAB source. The current conception is that formation of fragment ions is driven by interactions of myoglobin ions with high energy H_n^+ (n=1,2,3) ions.

A confusing effect is observed for the high m/z fragments, excluding the charge-reduced species at m/z~2200, which appear to be isotopically unresolved. It is not yet clear whether this effect can be attributed to the elevated pressure levels maintained during detection inside the orbitrap mass analyzer following injection of multiple gas pulses or whether this is a real chemical effect. **Figure 7** shows fragment ions in the high m/z range of the mass spectrum, highlighting the isotopically unresolved distributions.

Next steps in the development of the HAB source include testing further the current version of the system, which excludes the neutralization surface in order to understand and optimize the major dissociation pathways using a few standard protein ions, including proteins with disulfide linkages. These experiments will eventually be extended to antibodies.

The final step in this development process is the integration of the neutralization surface to the current configuration, as shown in **Figure 2** and **Figure 3** (**a**) above.

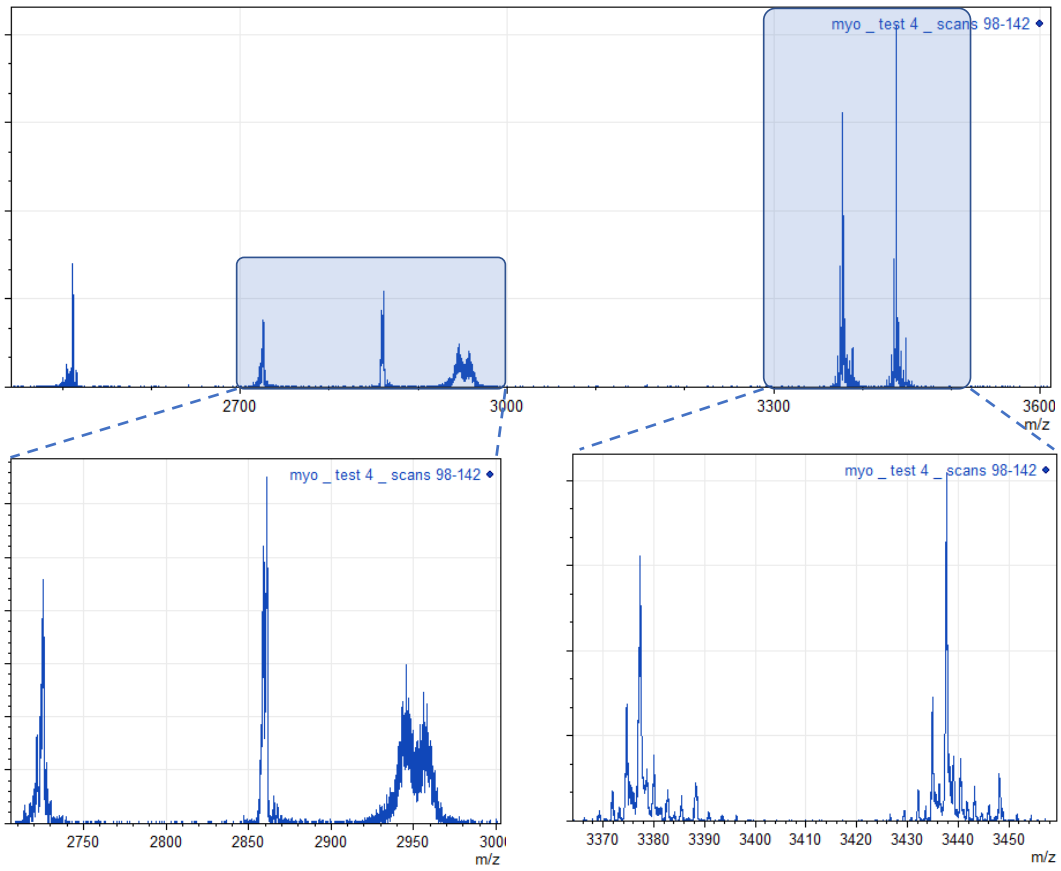

Figure 7. High m/z range of the MS/MS spectrum of $[M+9H]^{9+}$ ions of myoglobin irradiated with five consecutive gas pulses. The isotopically unresolved high m/z range fragment ions are highlighted.

Summary:

A working HAB source has been developed and top-down dissociation of myoglobin is demonstrated successfully. Multiple activation-dissociation pathways are observed and preliminary results are briefly outlined above. It is understood that fragmentation is initiated by hydrogen ions although contributions from hydrogen atoms or other metastable species entrained in the free jet expansion cannot be excluded. Extensive experimentation and data analysis of the HAB source in its current format is currently underway with smaller proteins while investigations will be extended to antibodies prior to integrating the neutralization surface.