

# Experimental and Computational Comparison of Collision Cell and In-Source Collisionally Activated Dissociation Kinetics in an IM-QTOF Instrument

Samantha O. Shepherd<sup>1</sup>, Ruwan T. Kurulugama<sup>2</sup>, Kenneth R. Newton<sup>2</sup>, and James S. Prell<sup>1</sup>



<sup>1</sup>Department of Chemistry and Biochemistry, University of Oregon, Eugene, OR <sup>2</sup>Agilent Technologies, Santa Clara, CA



## Introduction

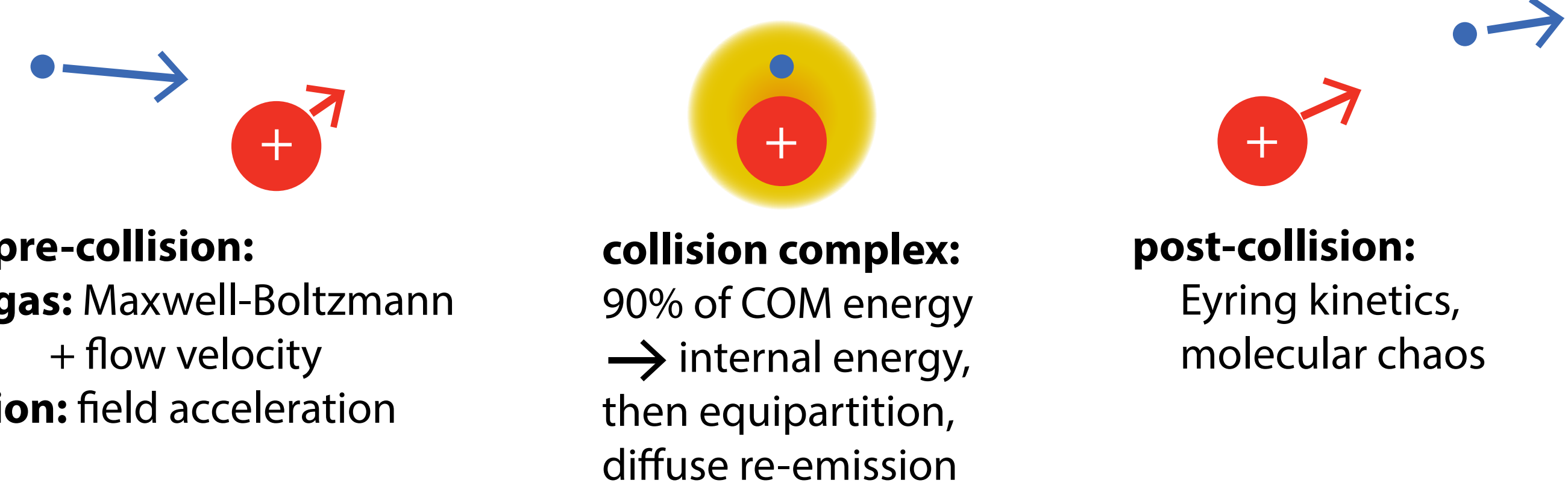
Understanding the mechanisms and energetics (including enthalpies and entropies) of gas-phase chemical reactions of ions is a cornerstone of informed experiment design and optimization. Dissociation/unfolding kinetics for ions ranging from simple organics to protein complexes depend on their internal energy and the duration of the observed process. However, gas pressures, density, flow velocity, and electric field profiles within ion activation regions can differ greatly between and within mass spectrometers. Our goal in this project was to solve this problem by explicitly modeling ion temperature as a function of time using gas and electric field input data.

While conventional Collision Induced Dissociation (and, for proteins, Unfolding<sup>1</sup>) takes place at relatively low pressure, as in the hexapole Collision Cell of the Agilent 6560 ESI-IM-Q-TOF instrument, a newly introduced source modification enables CID and CIU *before* ion mobility separation. Here, we combine gas dynamics simulations in Fluent, electric field simulations in COMSOL, and ion heating, cooling, and kinetics simulations in our home-built program IonSPA to model CID of Agilent Tune Mix ions in both the Collision Cell and modified source to determine consistent activation enthalpies and entropies and explore differences between the two activation regions.

## Methods

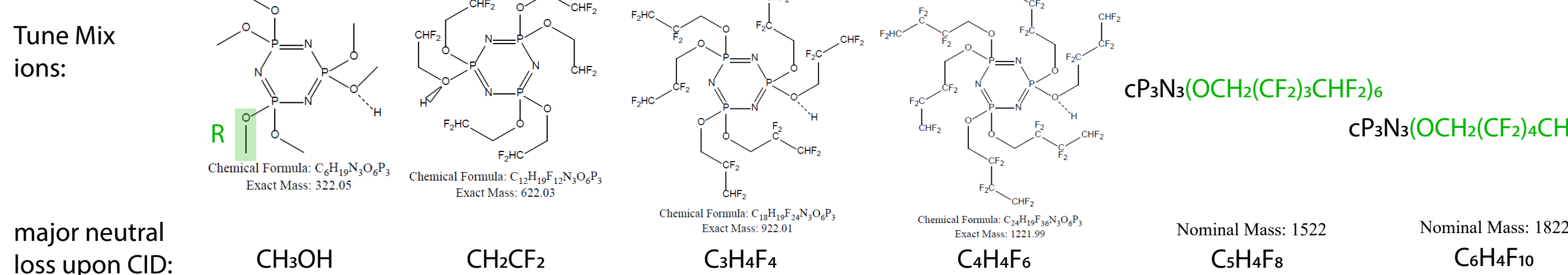
**IonSPA** (Simulations of the Physics of Activation) is our Python program which models ion heating and cooling based on our previously described protocol<sup>2</sup> with several major new additions for improved realism and accuracy (diffuse scattering, gas flow velocity field inputs, and electric field interpolation).

Ion dissociation in IonSPA is modeled using first-order Eyring kinetics. Initial guesses for  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  are based on coarse fits of experimental data (ln of rate constant vs. inverse modeled ion temperature), and these are further optimized by running a fully time-dependent temperature simulation of Eyring kinetics.



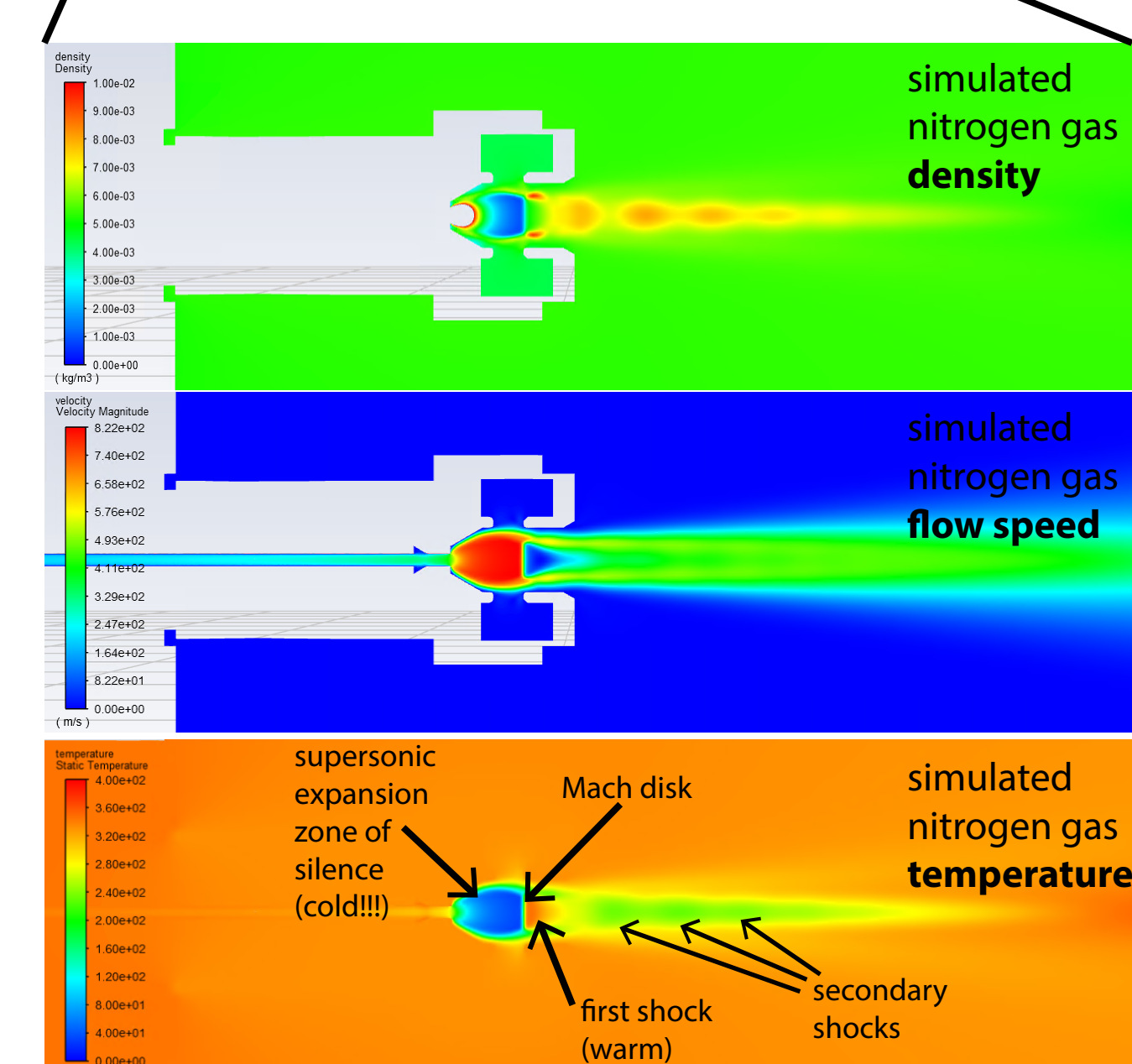
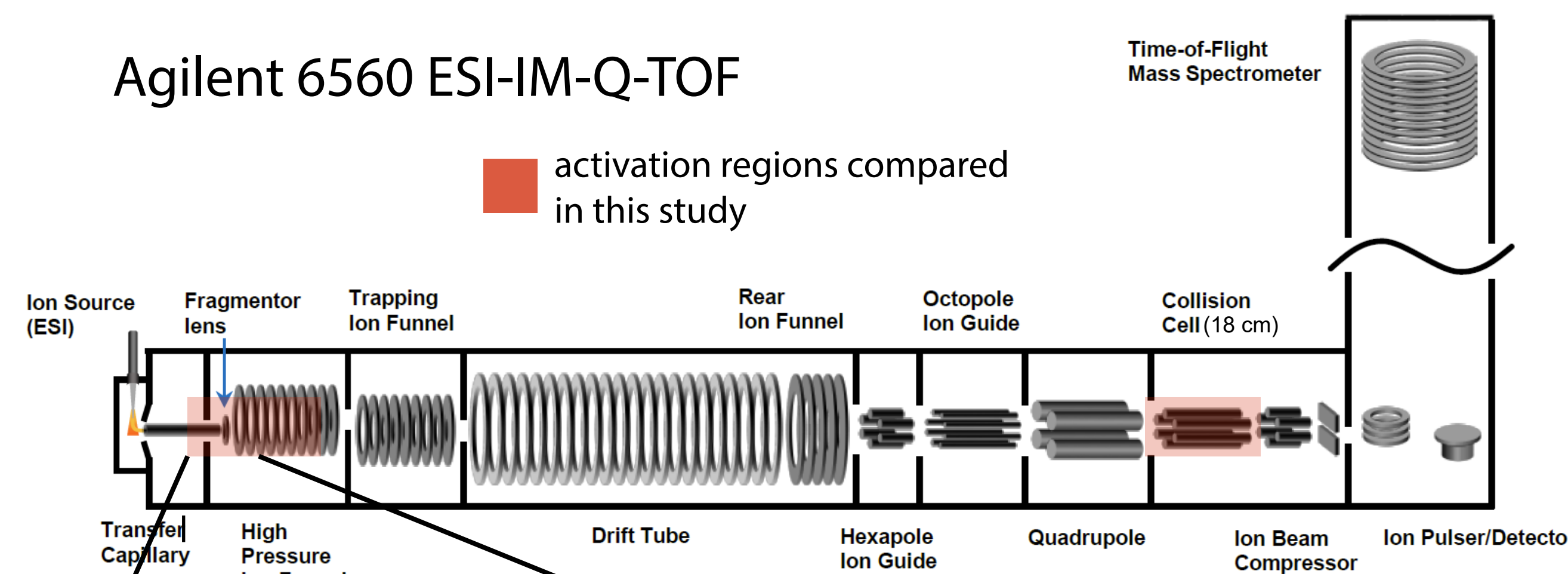
Electric potentials are simulated using COMSOL, and (nitrogen) gas dynamics are simulated using Fluent. Experimental data were acquired on an Agilent 6560 IM-Q-TOF instrument with a modified source design for increased range of ion activation. In this design, a potential of 0-450 V is applied across a small gap between the source Capillary Exit and the Fragmentor Lens, resulting in acceleration of ions through the supersonic expansion region and Mach disk. At high potentials, this results in rapid heating far in excess of ion temperatures encountered in the downstream Collision Cell, but for a few microseconds before rapid cooling.

Agilent Tune Mix ions were introduced via electrospray ionization from aqueous solution. Ions studied are protonated cyclotriphosphazine (cP3Ns) derivatives with nominal  $m/z$  322, 622, 922, 1222, 1522, and 1822.



## Results

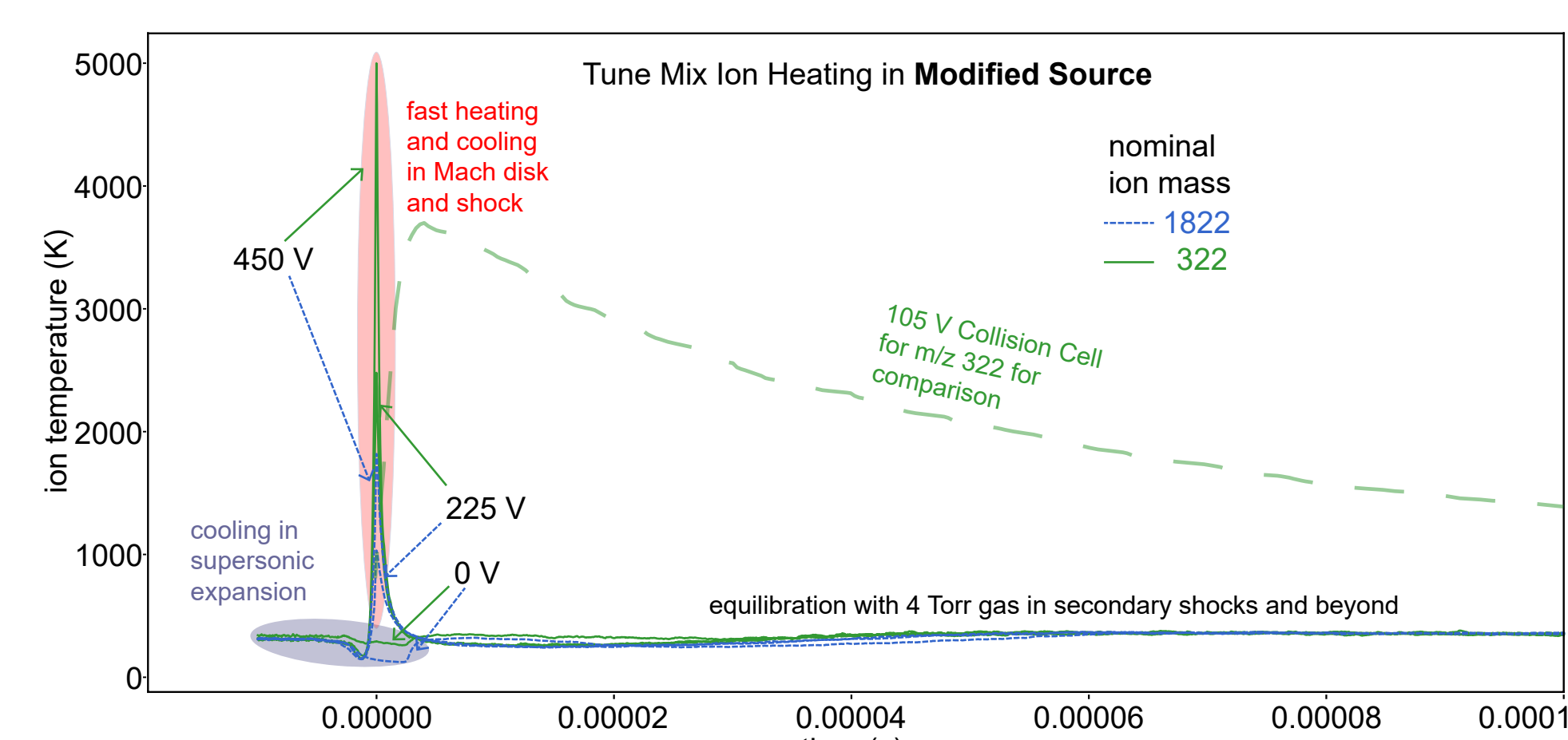
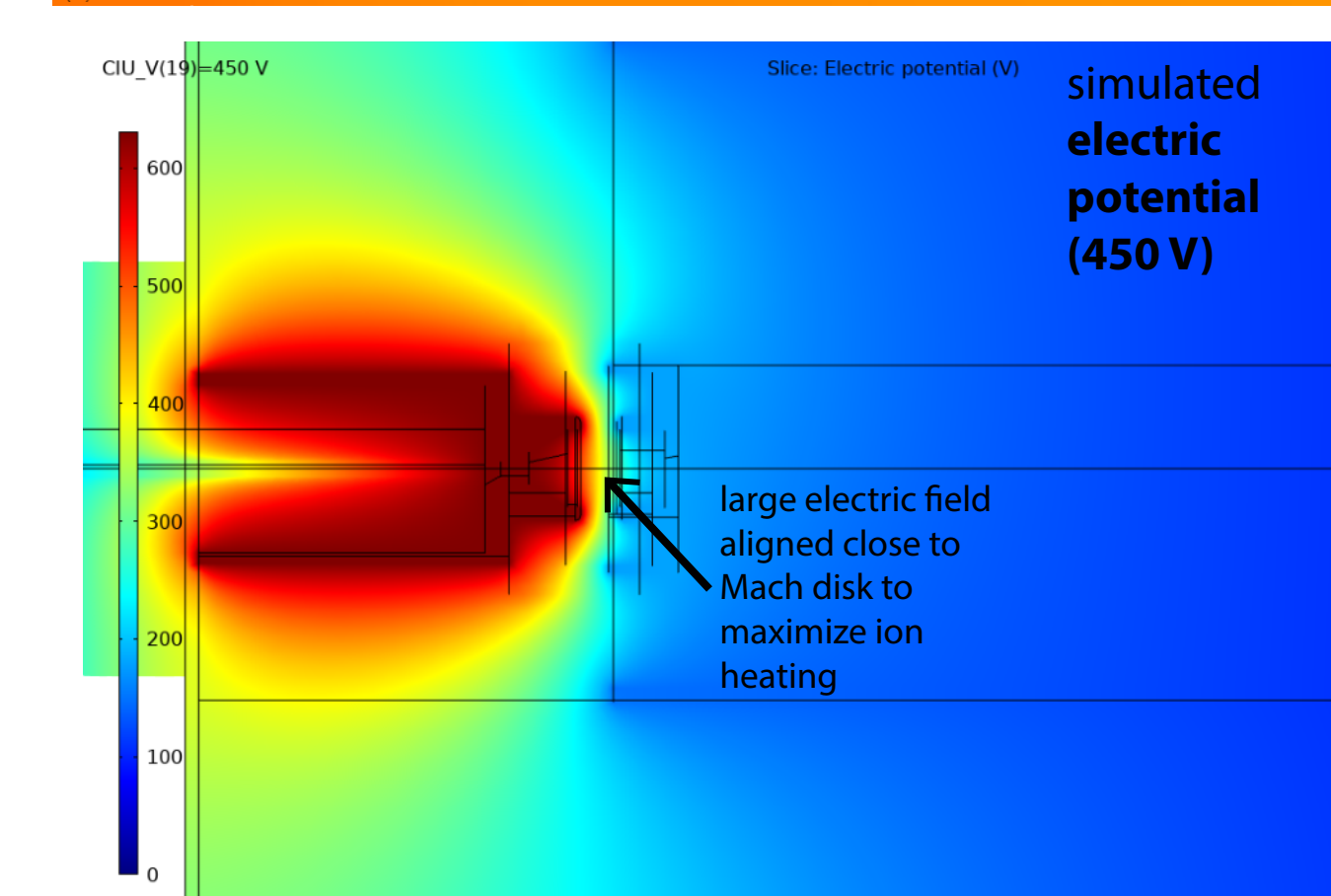
### Agilent 6560 ESI-IM-Q-TOF



In the modified source design, a 275-325 °C Source temperature and 4 Torr Front Funnel base pressure are used, with 0-450 V applied across a 1.4 mm gap between the end of the Transfer Capillary and the Fragmentor Lens.

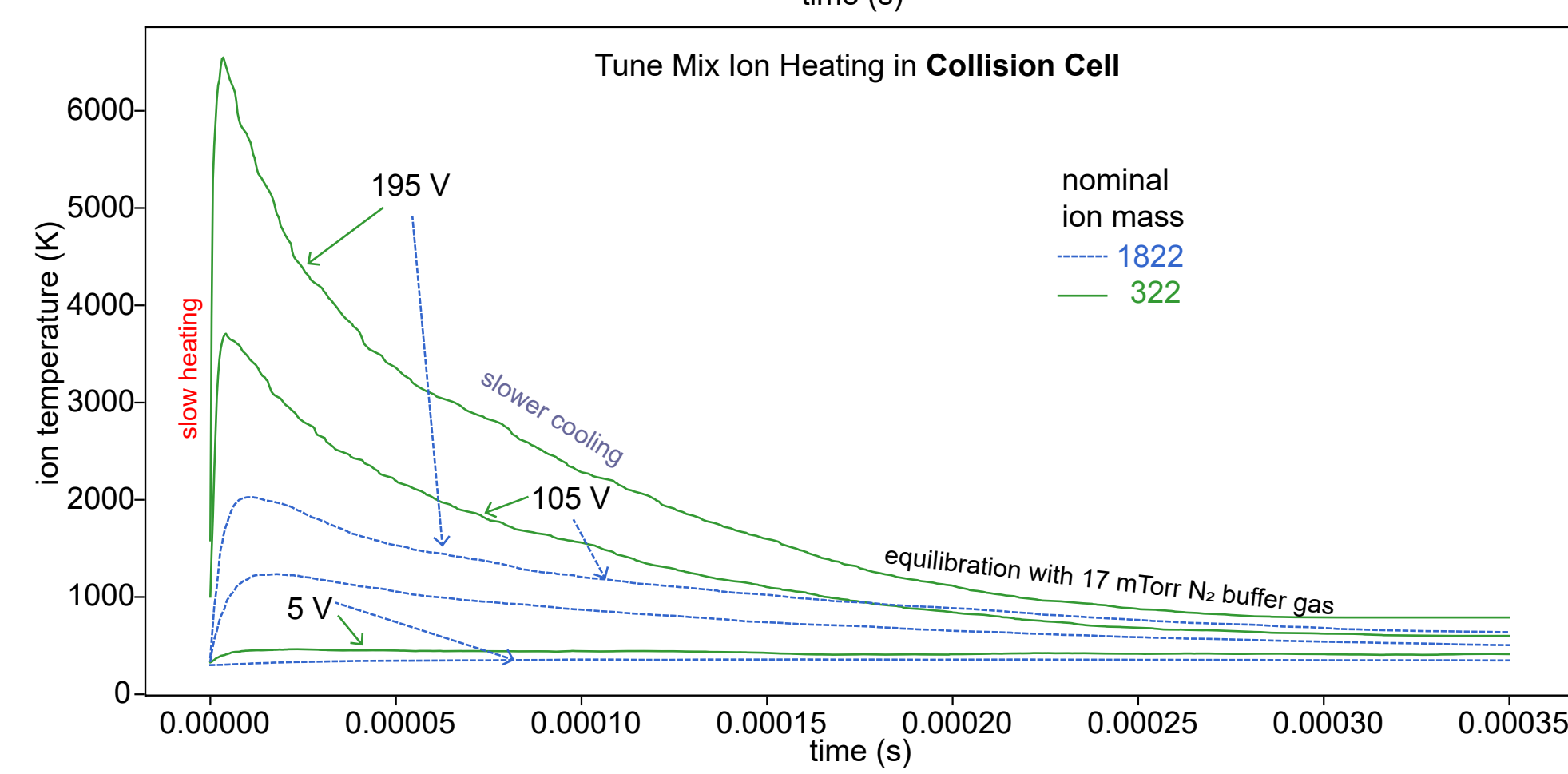
Fluent simulations illustrate the gas flow properties of the supersonic expansion, Mach disk, and secondary shock train within the Front Funnel. COMSOL simulations illustrate that the largest electric fields experienced by the ions coincide with the Mach disk. This is a key feature that allows ions to be heated rapidly over a very short distance.

Ions in the modified source quickly return to sub- or near-ambient temperatures as they progress through the secondary shocks and into the turbulent flow region of the Front Funnel.



IonSPA simulations of ion heating and cooling in both activation regions illustrate the differences in efficiency and timescale of the Tune Mix ions.

In the **modified source**, ions heat and cool over a ~5 microsecond timescale. In the conventional Collision Cell, heating occurs within ~10 microseconds, and cooling occurs much more slowly, over ~100 microseconds.



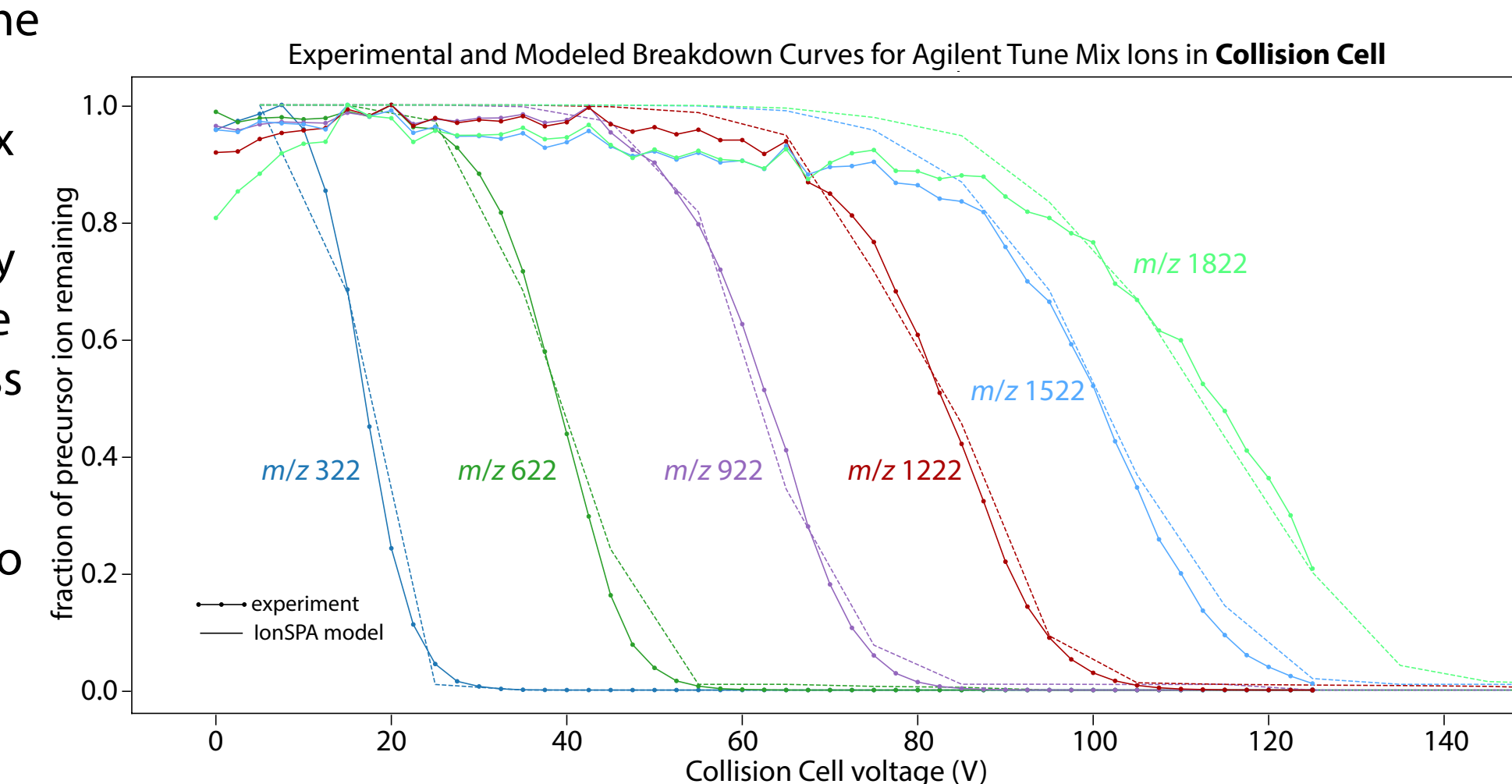
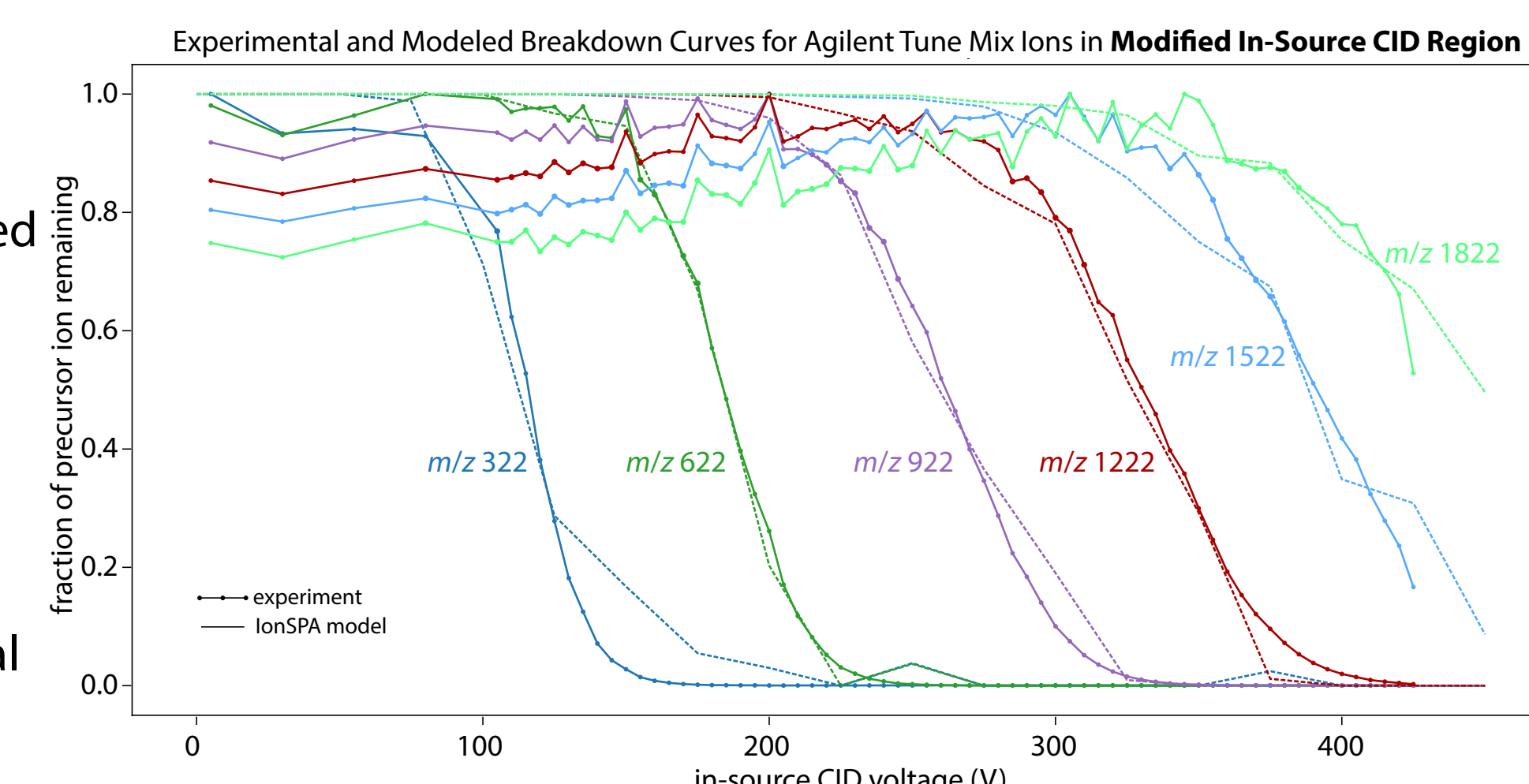
These differences allow for user choice between high temperatures for a short time vs. lower temperatures for a long time.

Initial guesses for activation enthalpy and entropy were based on experimental data, IonSPA ion temperature modeling, and our previously described Eyring kinetics fitting. These initial results were then used in fully time-dependent kinetics simulations in IonSPA for manual optimization of their values.

Excellent agreement between the IonSPA model and experiments was observed for all six Tune Mix ions studied, with only small differences in the fitted enthalpy and entropy of activation for the two different regions of the mass spectrometer. Given the very different timescales of ion heating and cooling in these two regions, these results strongly support the accuracy of the IonSPA model.

Modeled values for  $\Delta H^\ddagger$  indicate a mechanism consistent with simultaneous formation and cleavage of a bond at the transition state (e.g., rearrangement), and highly negative  $\Delta S^\ddagger$  support "tight" transition state for all ions studied that are also consistent with rearrangement reactions.

Overall, the activation energy and enthalpy values obtained from both methods using IonSPA are remarkably consistent.



Tune Mix ion ( $m/z$ )	in-source CID		conventional Collision Cell		average value	
	$\Delta H^\ddagger$	$\Delta S^\ddagger$	$\Delta H^\ddagger$	$\Delta S^\ddagger$	$\Delta H^\ddagger$	$\Delta S^\ddagger$
322	98	-52	69	-42	84	-47
622	99	-62	77	-62	88	-62
922	113	-65	98	-55	106	-70
1222	120	-65	105	-55	113	-70
1522	114	-70	93	-58	104	-64
1822	108*	-75*	34*	-60*	108	-75

\*Complete dissociation not achieved due to dielectric breakdown above 425 V for in-source design.

## Conclusions

Our program IonSPA can model time-dependent ion heating and cooling as well as corresponding first-order dissociation kinetics (using time-dependent temperatures) in a Synapt TWAVE Trap and in an Agilent 6560 hexapole Collision Cell and modified in-source CID region, requiring minimal set of user input (ion collision cross section, mass, charge, number of atoms) after electric fields and gas flow properties have been sufficiently specified.

Results for Agilent Tune Mix ions indicate CID barrier thermochemistry consistent with rearrangement reactions, as expected from known neutral losses. These enthalpy and entropy values illustrate powerful mechanistic insights that can be gained from this type of study.

Future plans include incorporation of ion size- and temperature-dependent heat capacities in IonSPA and streamlined uploading of results from field modeling and fluid dynamics programs.

**Acknowledgments:** The National Eye Institute (R01-EY027012), and Prof. Jody May for ion structures.

**References:** <sup>1</sup>Ruotolo and coworkers, *Anal. Chem.* **2020**, *92*, 15489. <sup>2</sup>Prell and coworkers, *Chem. Sci.* **2019**, *10*, 4097. <sup>3</sup>JASMS **2020**, *31*, 602. <sup>4</sup>*Anal. Chem.* **2020**, *92*, 10365.