

# Systematic studies of the impact of chemical modifiers on the ion population observed with nESI MS



Physical & Theoretical Chemistry

Wuppertal, Germany

Institute for Pure and Applied Mass Spectrometry

Marco Thinius; Christine Polaczek; Clara Markert; Duygu Erdogan; Hendrik Kersten; Thorsten Benter

## Introduction

Currently, there is no comprehensive model of the electrospray ionization process that explains all experimental observations. New developments, as for example the Bruker nanoBooster™ technology, strongly suggest that cluster chemistry is playing an important role in ESI, as it does in virtually all API techniques.

In the interface region between the ion source and the analyzer of an API mass spectrometer the combination of strong electrical fields (leading to high effective ion temperatures), the presence of a variety of reactive compounds, and sufficiently high collision rates implies that the primarily generated ion population “within the ion source” will be affected when traveling through this “chemical reactor” [1].

### Supercharging

The formation of highly charged ions (e.g. protonated proteins) is crucial for MS/MS experiments as it improves the efficiency of selective fragmentation methods. Many studies have shown that certain substances (superchargers) increase the average charge state of multiply charged ions when added either to the analyte solution or the background gas of the ion source (the latter species are also referred to as chemical modifiers). Experimental observations suggest that there is a more general mechanism behind those two approaches towards “supercharging”. Within this mechanism, “supercharging” is not the addition of charges, but rather the result of charge-conservation [2].

### Heterogeneous processes

Heterogeneous processes, e.g., at liquid-gas interfaces (droplet surface) will most likely play an important role in the ESI processes. The importance of considering solid surface interactions came recently into focus upon the introduction of “inlet” ionization [3]. Thus, the choice of inlet stages for API MS (e.g. transfer capillary, nozzle) may become an important parameter for instrument operation as well. These processes however are not taken into consideration in the present contribution.

## Methods

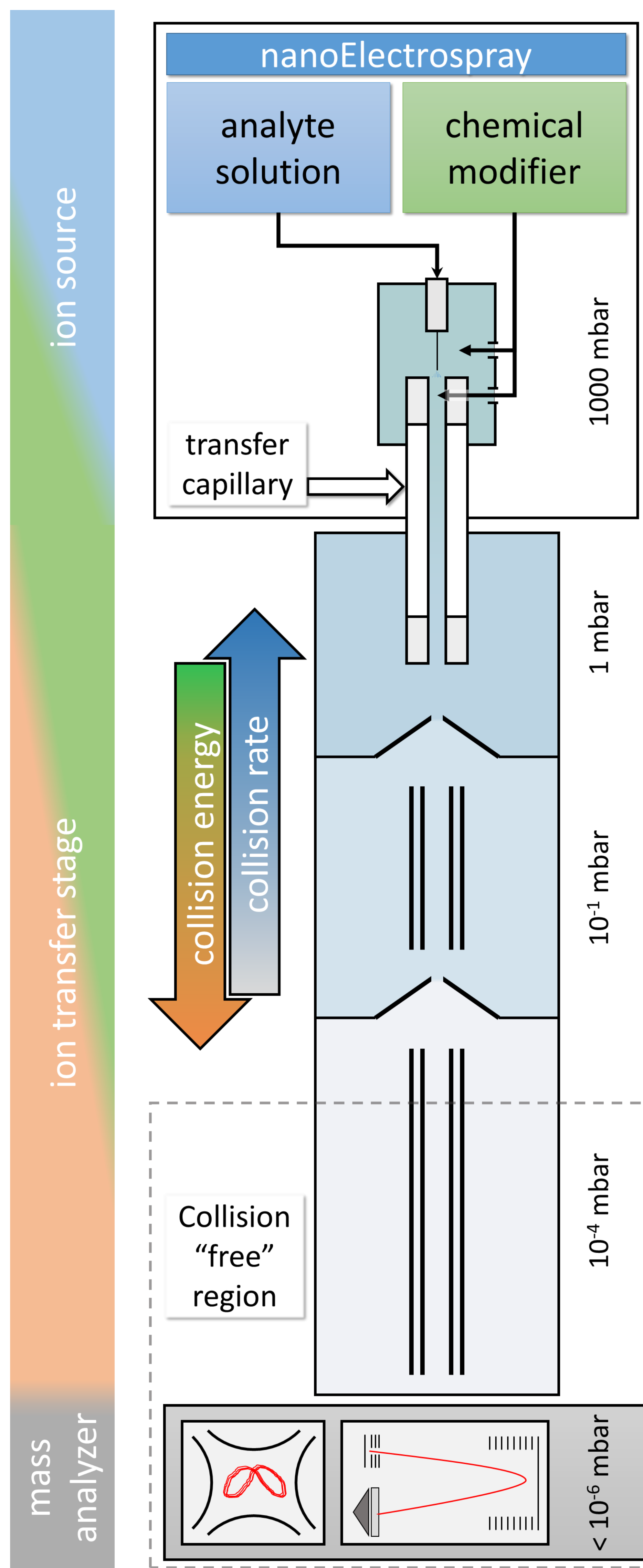
**MS:** micrOTOF, amazon Speed ETD, esquire 6000 (all Bruker Daltonics, Bremen, Germany)

**Ion Source:** custom nano Electro Spray Ion (nESI) Source [2]; Bruker CaptiveSpray nanoBooster™ (Bruker Daltonics, Bremen, Germany)

**Gas Supply:** Nitrogen 5.0 (Messer Industriegase GmbH, Germany). All gas flows are controlled by mass flow controllers (MKS Instruments, Germany)

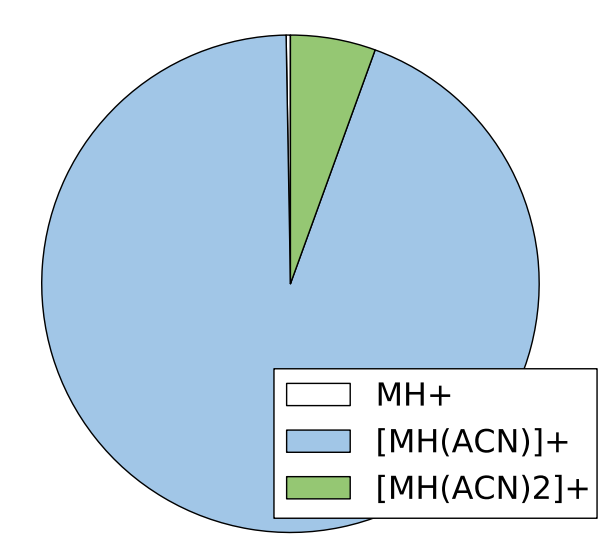
**Chemicals:** all chemicals were purchased from Sigma Aldrich, Germany, and used without further purification

## Experimental



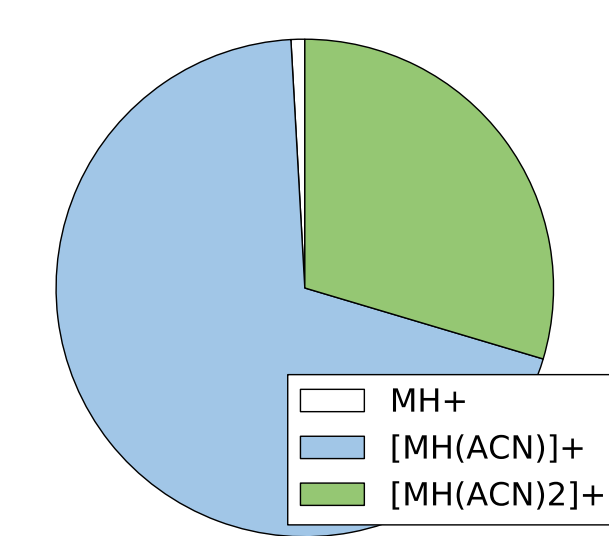
### Cluster formation via analyte solution only

nESI mass spectra of primary amines diluted in 50 % aqueous acetonitrile solution with 0.1 % formic acid show  $[M(ACN)H]^+$  as most abundant signal. The relative abundance of the observed species  $MH^+$ ,  $[M(ACN)H]^+$  and  $[M(ACN)_2H]^+$  is depicted for n-butylamine. Similar observations were made with aniline and n-hexylamine.

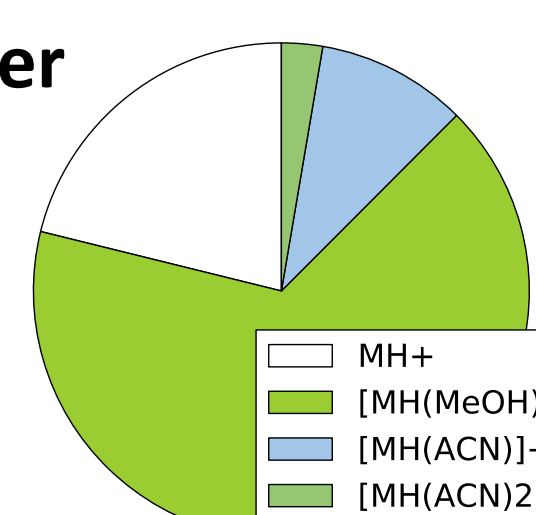


### Cluster formation via chemical modifier

Addition of ACN to the *gas phase* enhances the formation of  $[M(ACN)_2H]^+$ , shown for n-hexylamine.

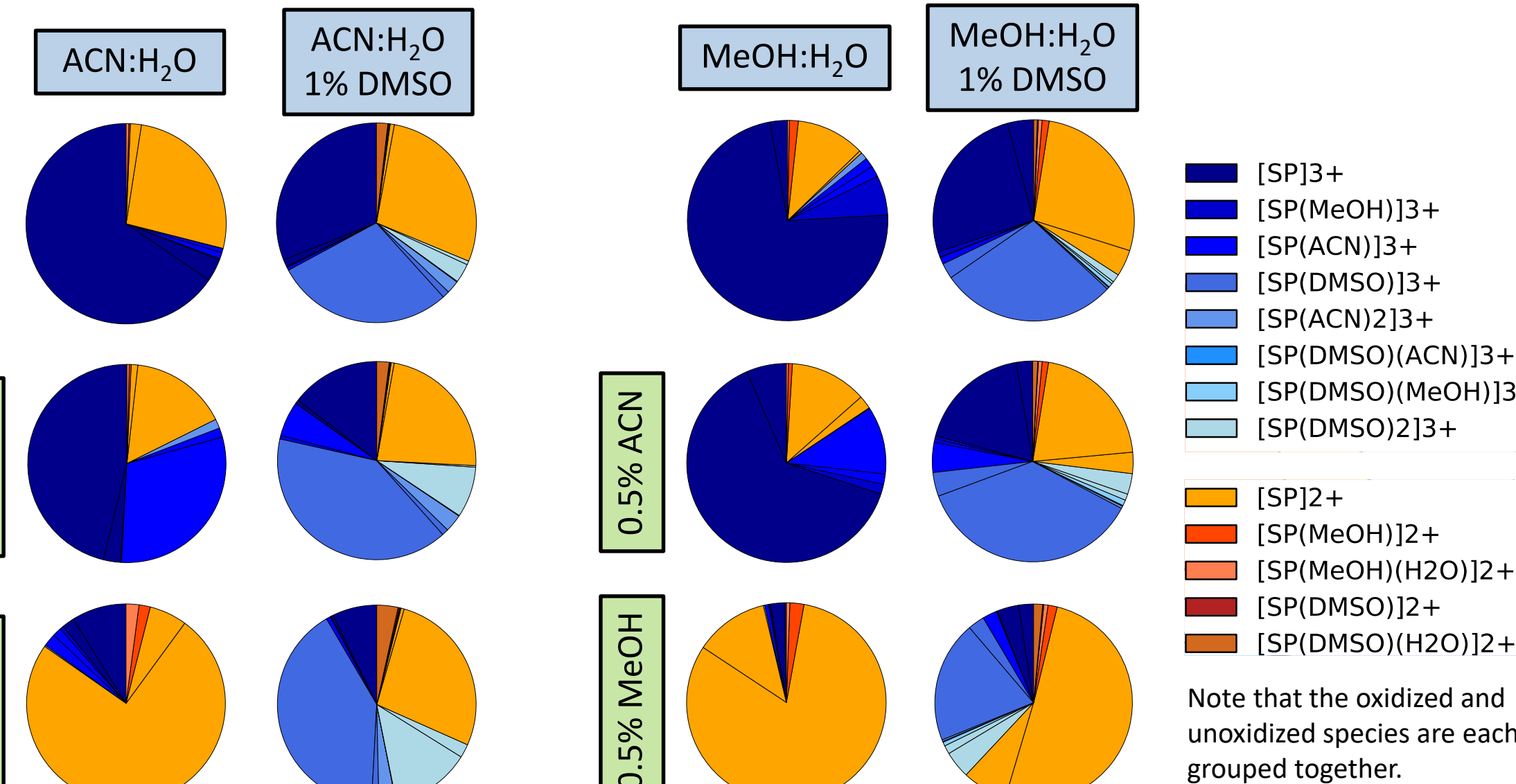


In addition to ACN containing clusters the MeOH adduct is detected when MeOH is added to the gas phase.



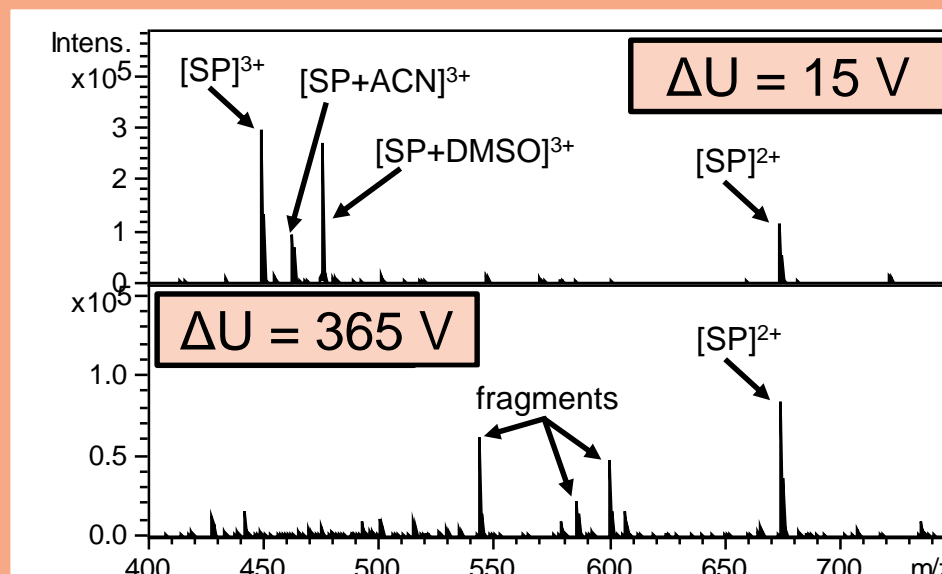
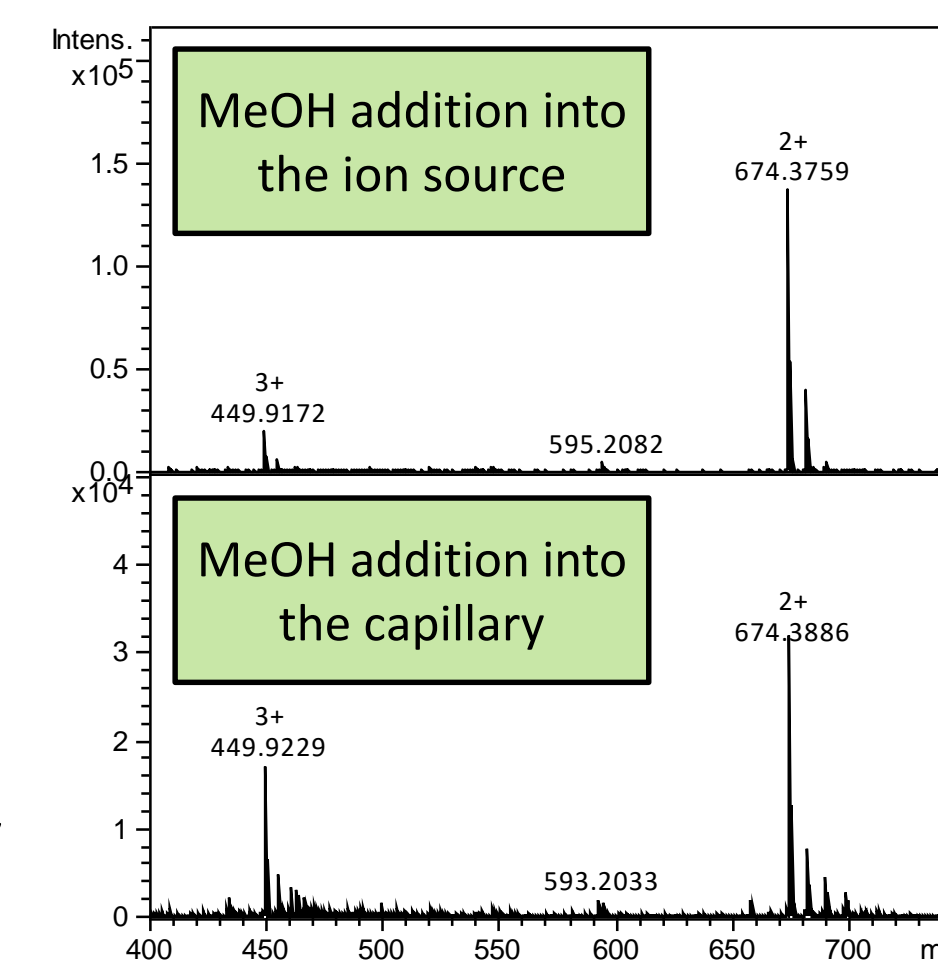
### Mixed clusters: chemical modifier and supercharger

nESI mass spectra of substance P (SP) show doubly and triply protonated species. Experimental investigations with different analyte solution and gas phase compositions, respectively, reveal the extent of change in the average charge state distribution. Depending on the present compounds, various cluster species are observed. In contrast to the primary amines shown above, the ACN adduct  $[SP(ACN)]^{3+}$  is generated to a certain extent only if **ACN** is added as a chemical modifier to the *gas phase*. Thus, gaseous ACN increases the average charge state. In contrast, MeOH as chemical modifier strongly depletes  $[SP(ACN)]^{3+}$  in favor of the doubly protonated species. This significant shift in the charge state ratio is prevented by addition of **DMSO** to the analyte *solution*. The pronounced generation of  $[SP(DMSO)_n]^{3+}$  clusters conserves the higher charged species. These effects are largely unaffected by the composition of the analyte solution (aqueous ACN vs MeOH). However, MeOH as solvent decreases the absolute signal intensities.

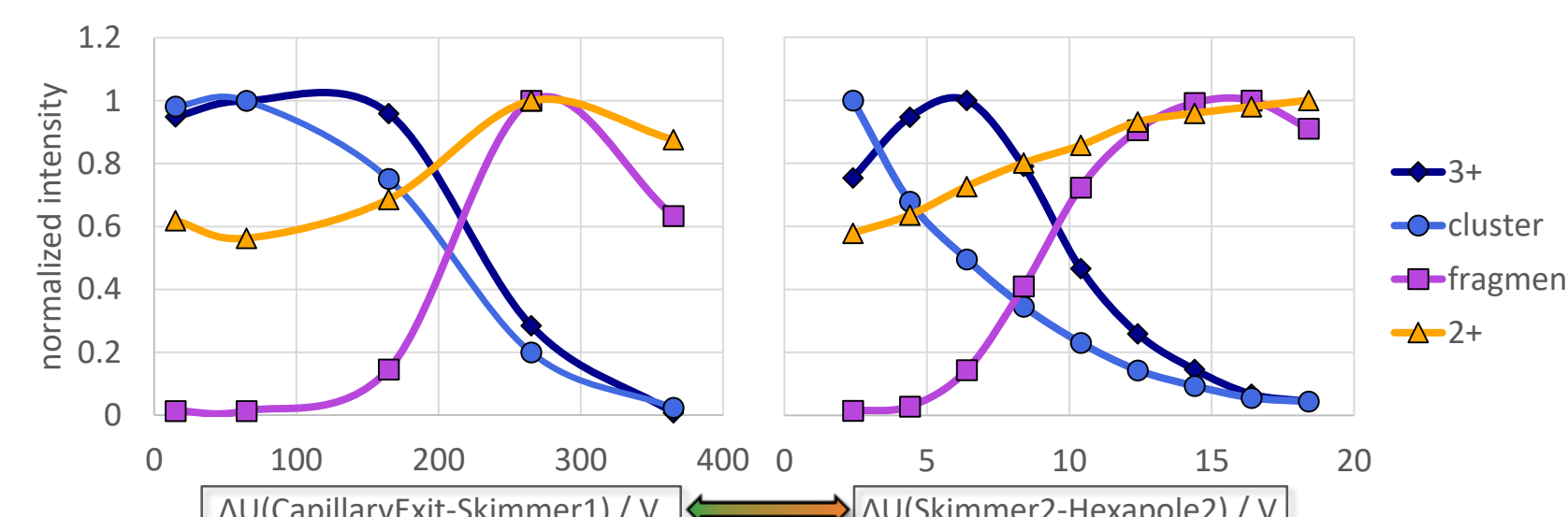


### Mixed chemical modifiers

Simultaneous addition of gaseous ACN and MeOH in varying mixing ratios reveals the competitive situation regarding the charged species and charge state distribution, respectively. It is found that the amount of ACN present determines the ratio between the unclustered and clustered triply protonated species, almost independently of the MeOH mixing ratio. The latter strongly affects the ratio between triply and doubly protonated species. Changing the reaction time by choosing a different entry port for the modifier (cf. left figure, source region) strongly impacts on the ion chemistry: Adding only MeOH via the capillary entrance leads to cluster formation with the doubly as well as the triply protonated species, which were otherwise never detected before.



**Decustering and fragmentation**  
CID in the transfer stage leads to decustering and fragmentation of the triply protonated ion, the small increase of the doubly protonated ion signal may be attributed to charge loss during the forced decustering process of  $[SP]^{3+}$ .



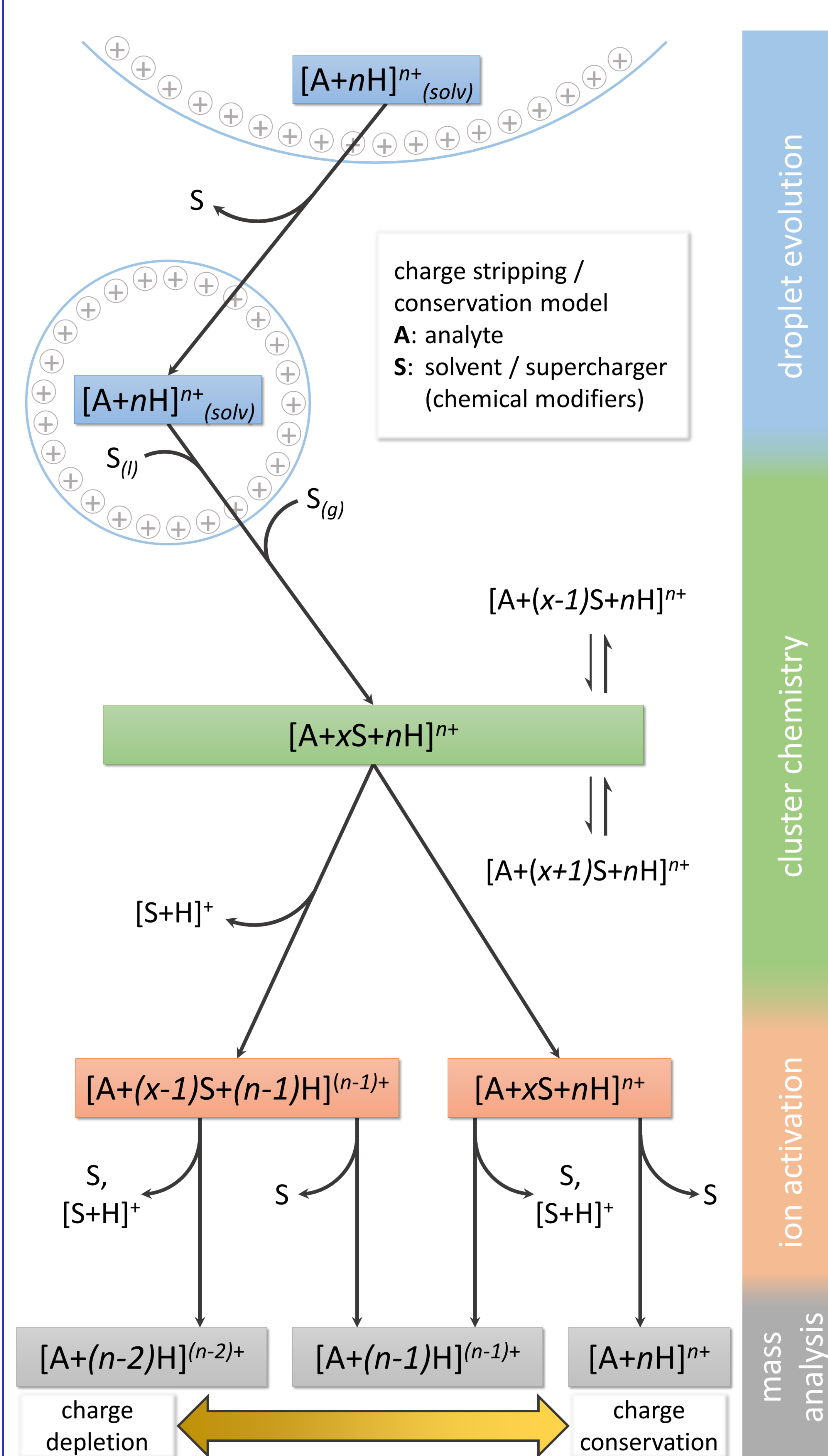
## Literature

- [1] S. Klee, A. Brockhaus, M. Thinius, W. Wissdorf, T. Benter, Fundamental ion-molecule reaction studies at elevated ion temperature and analytical application of an ion activation stage (“ion tunnel”), *Proceedings of the 61th ASMS Conference on Mass Spectrometry and Allied Topics*, Baltimore, MD, USA (2014)
- [2] M. Thinius, M. Langner, H. Kersten, T. Benter, Impact of chemical modifiers on the cluster chemistry during electrospray ionization, *Proceedings of the 63th ASMS Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX, USA (2016)
- [3] S. Trimplin, “Magic” Ionization Mass Spectrometry, *J. Am. Soc. Mass Spectrom.* 27, 4–21 (2016)

## Summary / Outlook

- This study of combined solution and gas phase modifiers strongly promotes the hypothesis that in both cases clustering processes are responsible for charge conservation/depletion
- Preliminary investigations as well as the presently performed experiments confirm and augment the postulated cluster mechanism
- Further experimental and theoretical studies regarding the stability of the different cluster species are underway
- Heterogeneous processes and their role in ionization processes will be investigated in future experiments

## Mechanism



## Acknowledgement

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