A new fingerprinting of the tridimensional structure of macro-biomolecules applicable as a quality control criterion through unfolding pathways explored in gas phase.

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Introduction

This research project explores **collision-induced unfolding (CIU)** as a potential **quality control (QC)** method for industries, particularly pharmaceuticals. Traditional **structural analysis methods**, such as X-ray crystallography and NMR, are **effective but limited** by complex sample preparation and long experiment times. In contrast, **CIU**, coupled with **ion mobility-mass spectrometry**, provides a faster, more efficient alternative by mapping **energy landscapes** through biomolecular **structural transitions** in the gas phase. The project aims to deepen the understanding of how **various activation methods** affect these **structural changes**, while also **standardizing CIU results** to make it a reliable tool for pharmaceutical **QC**.
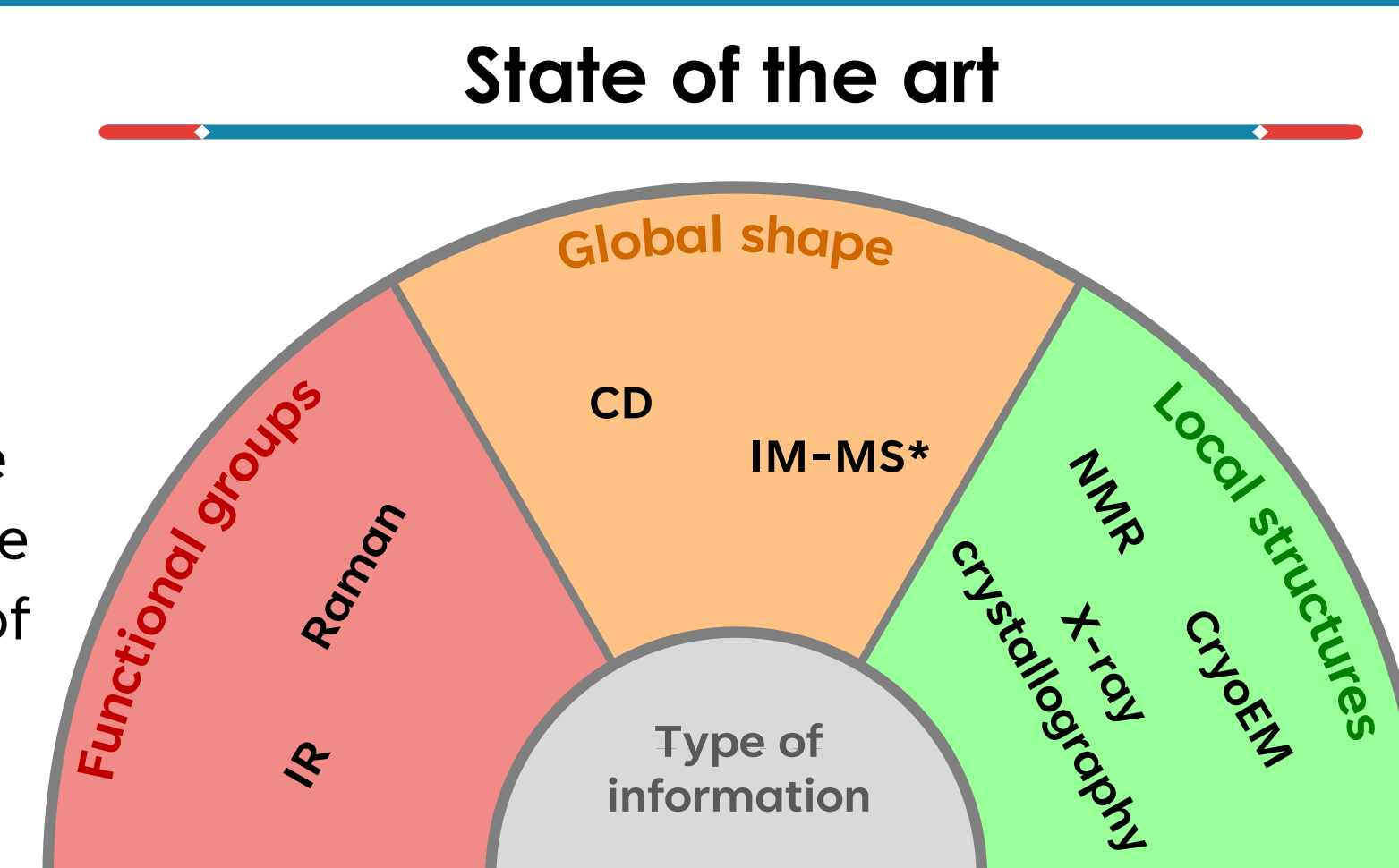
Ion mobility

- Separates **ions** based on their **charge** and **collision cross section (CCS)**.
- A **drift time** is measured and can then be used to obtain the **CCS**.
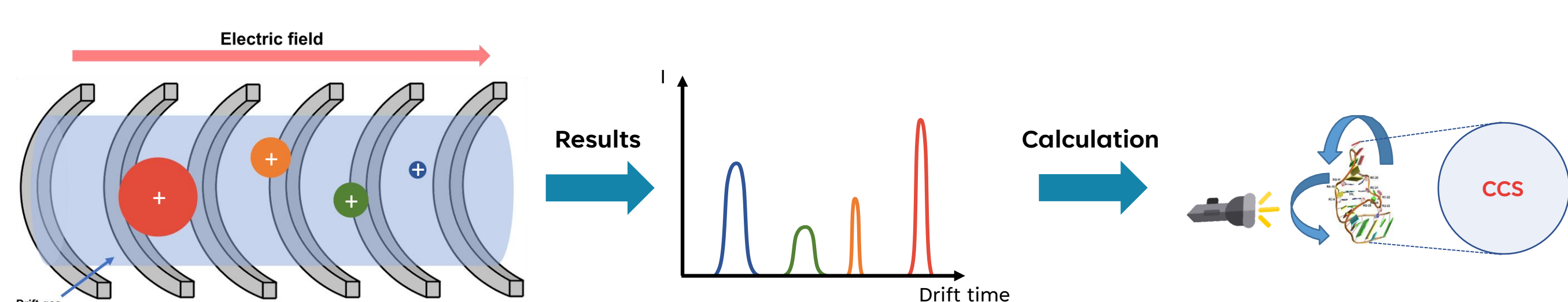

Context

State of the art

Classical methods tend to have some **drawbacks** (sample preparation, time of analysis,...)

* Compatible local structures could be obtained after theoretical calculations

Objectives and models

- Gather more information in complement to IM-MS by subjecting the models to **different activation methods**
- Study of the **applicability** of **collision-induced unfolding (CIU)** as a gas phase **quality control** method
- The **models** are biomolecules of **pharmaceutical interest** which adopt a **3-dimentionnal structure**

Proteins   Oligonucleotides

Protein-ON complexes
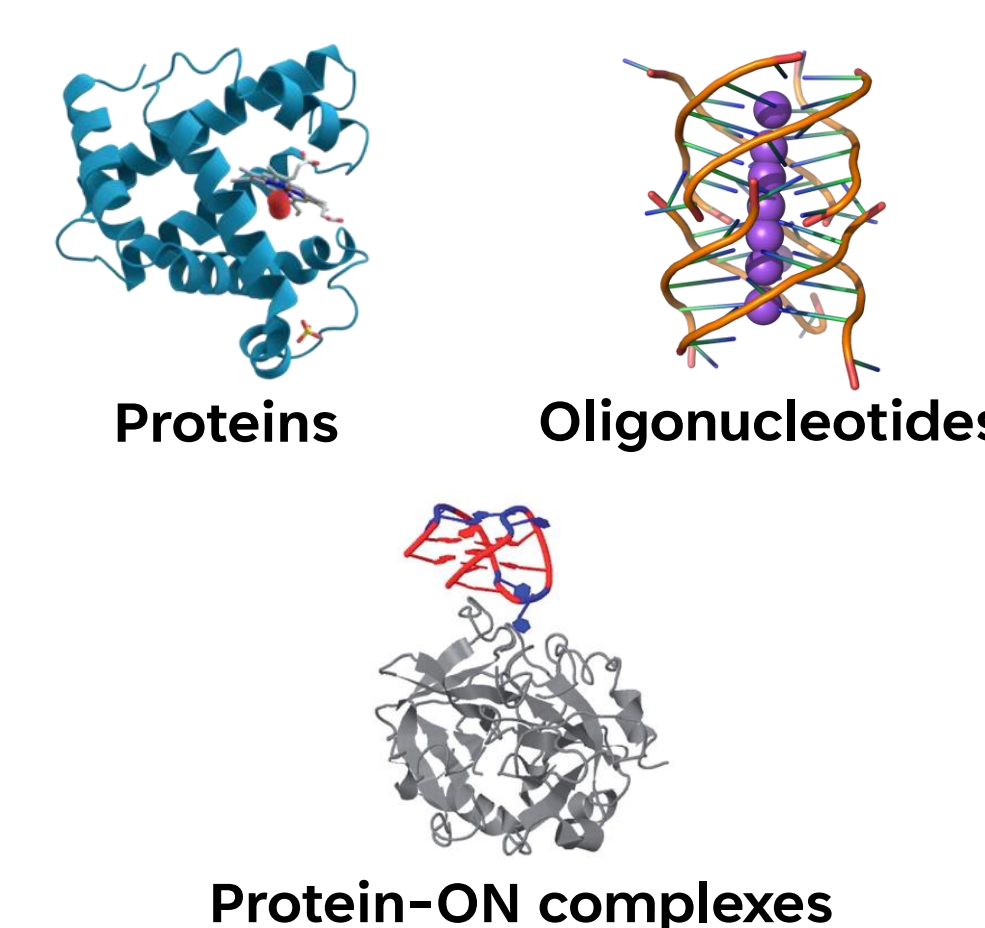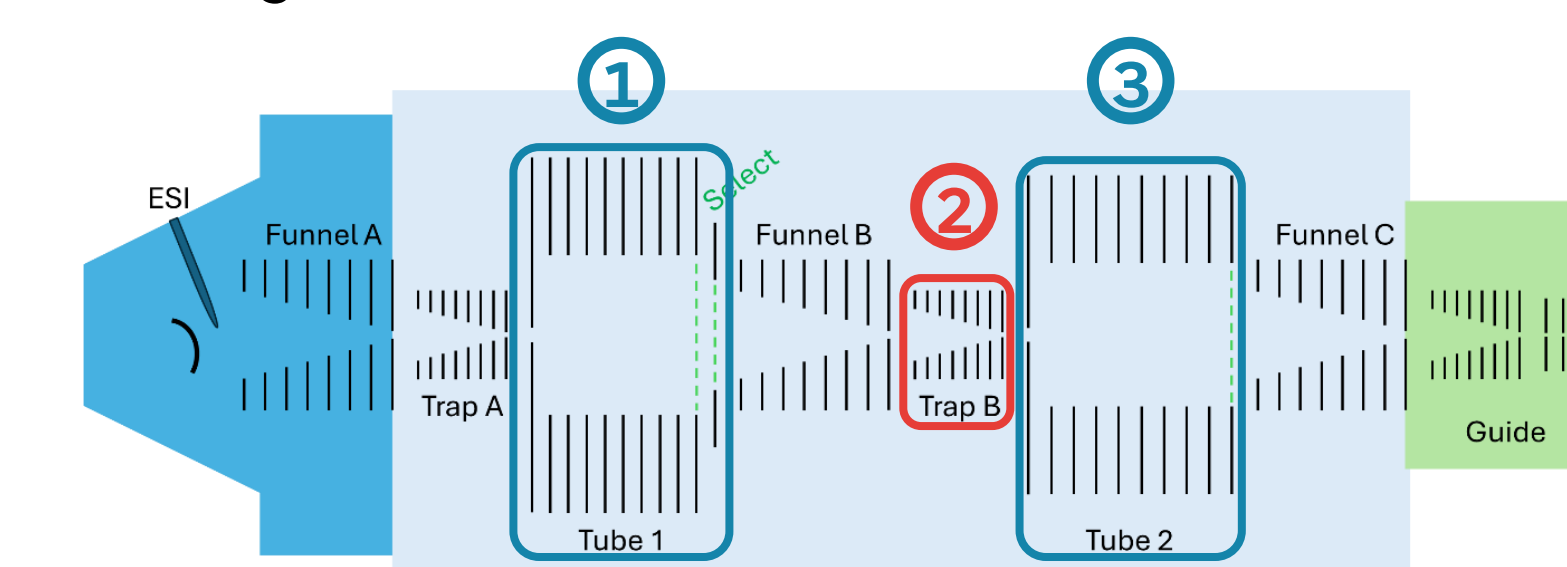
Activation methods

Collision-induced unfolding

- Energy-resolved **collision** experiments monitored by **IMS**
- The aim is to induce a **structural change**, **not fragmentation**
- Multiple **parameters** will be modified to assess their **impact**:
  - Type of gas (N_2, He, Ar,...)
 - Gas pressure
  - Trapping time
  - Acceleration voltages
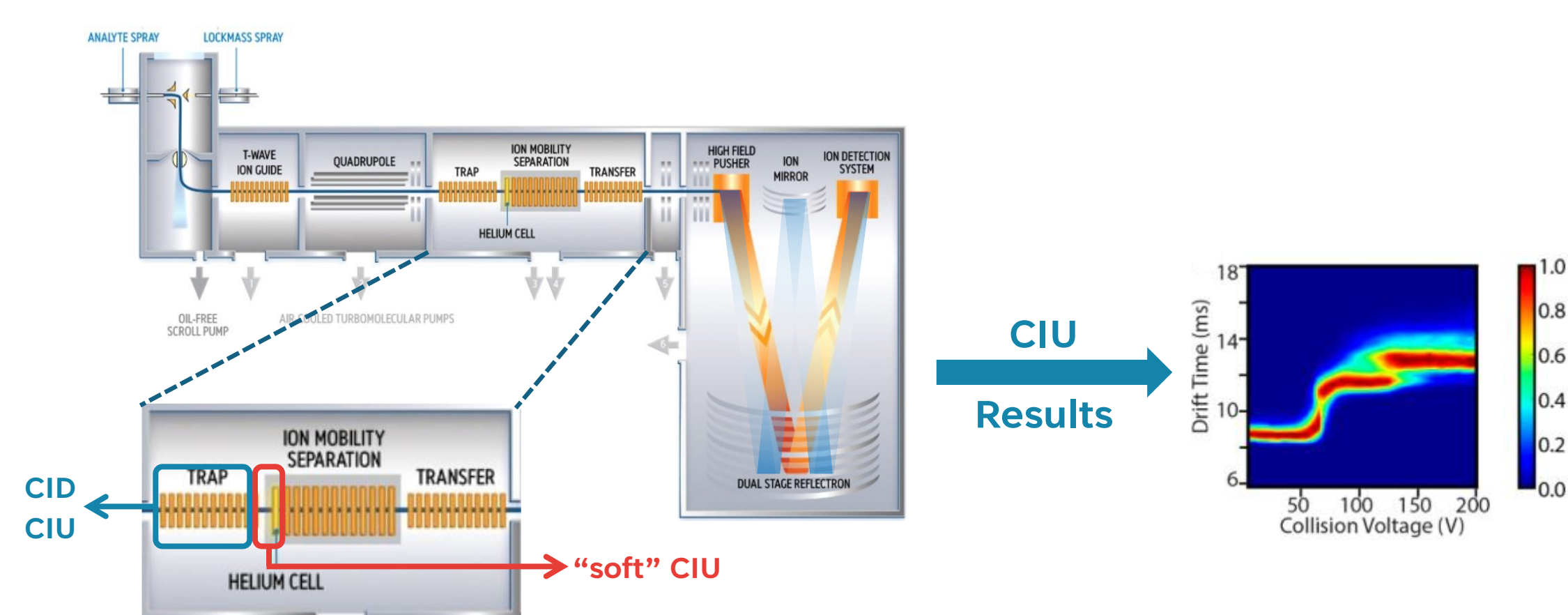
Alternative activation methods

- Utilization of a **tandem IMS** equipment for IMS monitoring after **alternative activation methods**
  - ① Selection of ions by their mobility
  - ② Activation: IR irradiation or heated gas
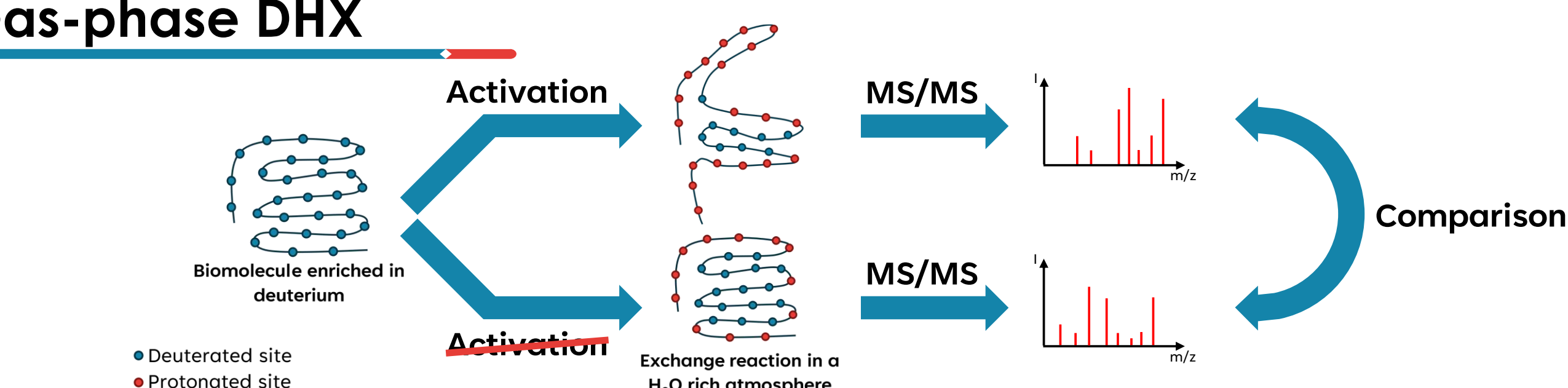 - ③ IMS analysis of the activated ions
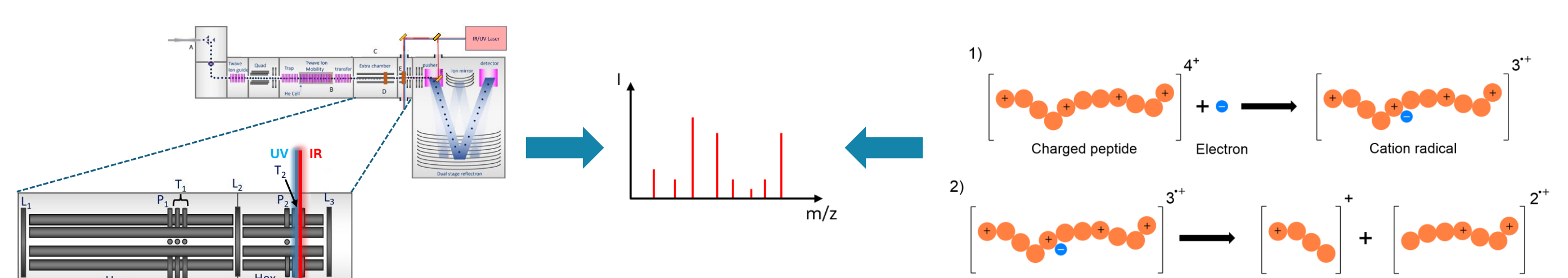

Preliminary results

✔ **IR** managed to display **differences in the spectra** upon the **loss of cations or a strand**
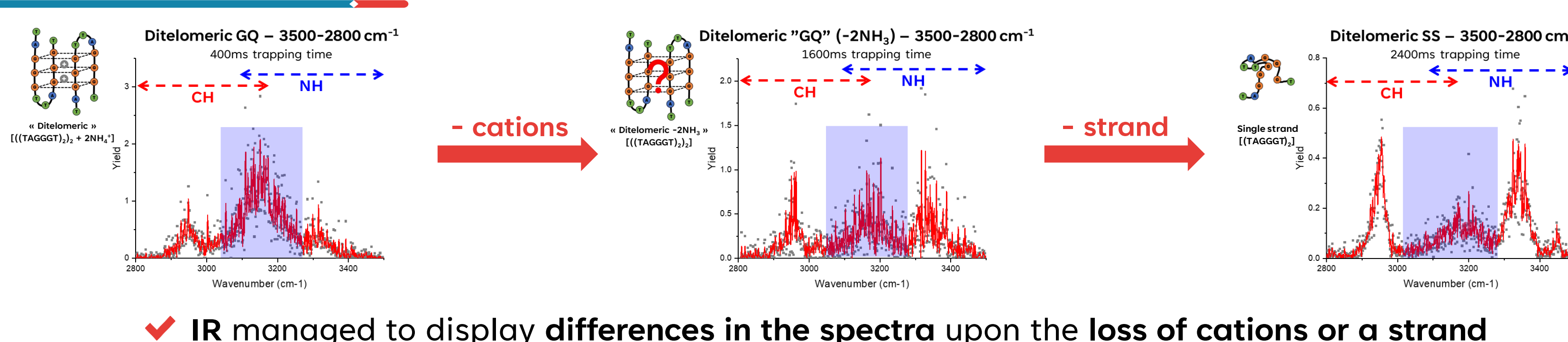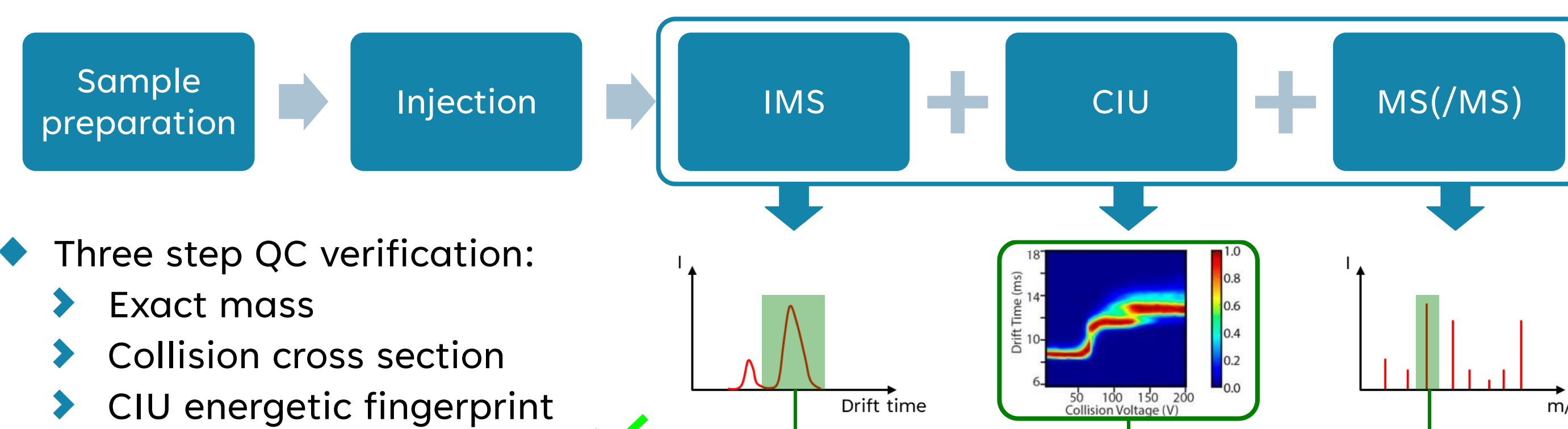
Further investigations

Gas-phase DHX

Non-ergodic fragmentation

- Utilization of **non-ergodic** fragmentation methods to investigated the **affected areas**
  - Ultraviolet photodissociation (UVPD)
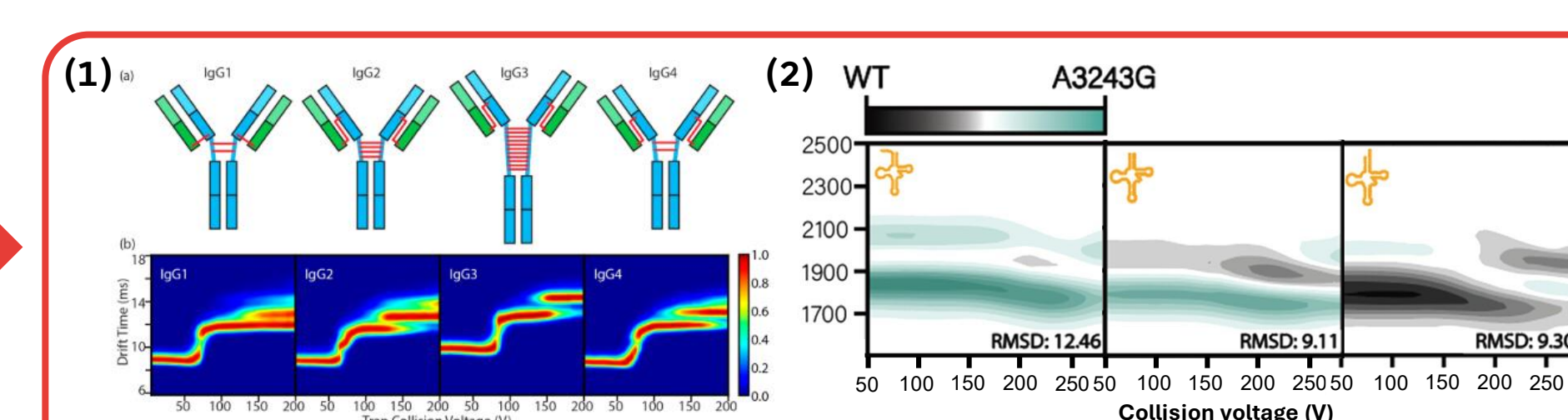 - Electron capture dissociation (ECD)

Application

- Investigation of the **applicability** of **CIU** as a reliable **quality control** step

- Three step QC verification:
  - Exact mass
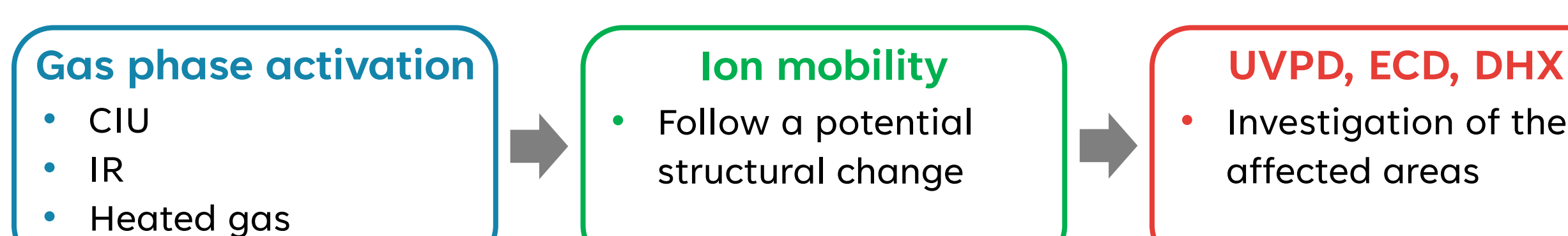 - Collision cross section
  - CIU energetic fingerprint

✔ Efficiency proven to discriminate similar structures (mAb, pAb, tRNA)

Conclusion

- Expected outcomes:
  - ✔ Better understanding of the fate of the injected energy when using different methods
  - ✔ Obtain some information about the energetic landscapes of the models
  - ✔ Development of a novel quality control step allowing for differentiation of highly similar structures

IM-MS workflow for biomolecule characterization

Gas phase activation
- CIU
- IR
- Heated gas

Ion mobility
- Follow a potential structural change

UVPD, ECD, DHX
- Investigation of the affected areas

Contact and partners information

References: (1) Tian, Y.; Han, L.; Buckner, A. C.; Ruotolo, B. T. *Anal Chem* **2015**, *87* (22), 11509–11515, **(2)** Anders, A. G.; Tidwell, E. D.; Gadkari, V. V.; Koutmos, M.; Ruotolo, B. T. *J Am Chem Soc* **2024**, *146* (7), 4412–4420.