Hyphenation of Ion Mobility Mass Spectrometry and Action Spectroscopy to Probe Structure and Kinetics of Peptide Aggregation

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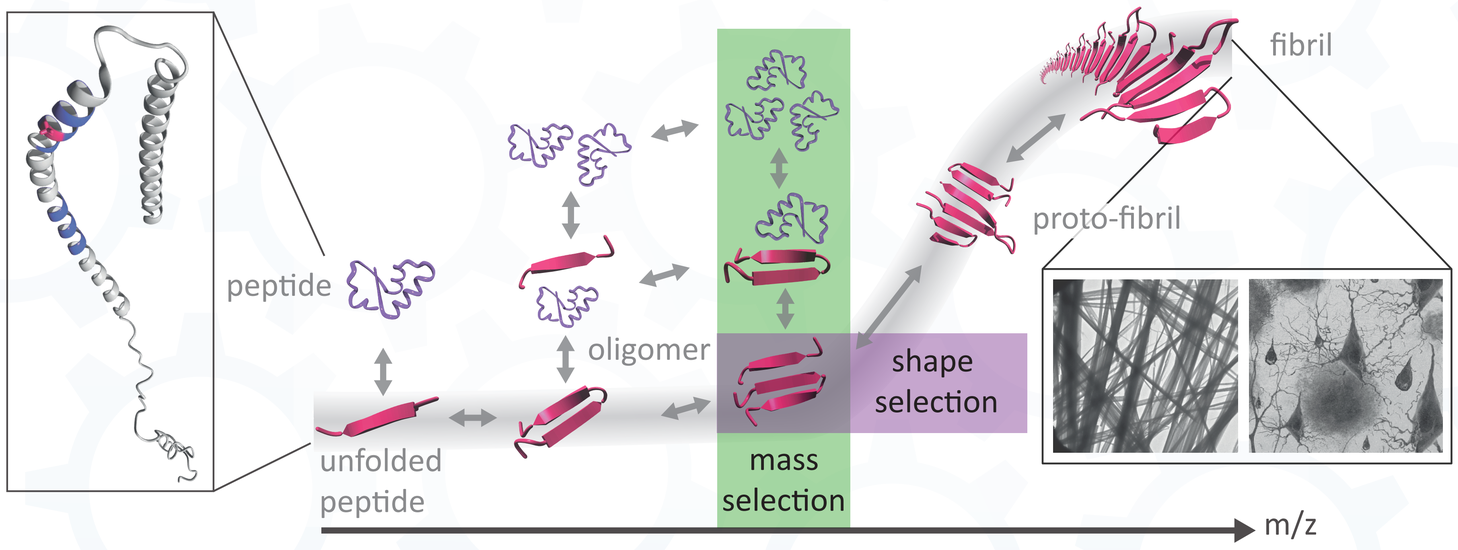
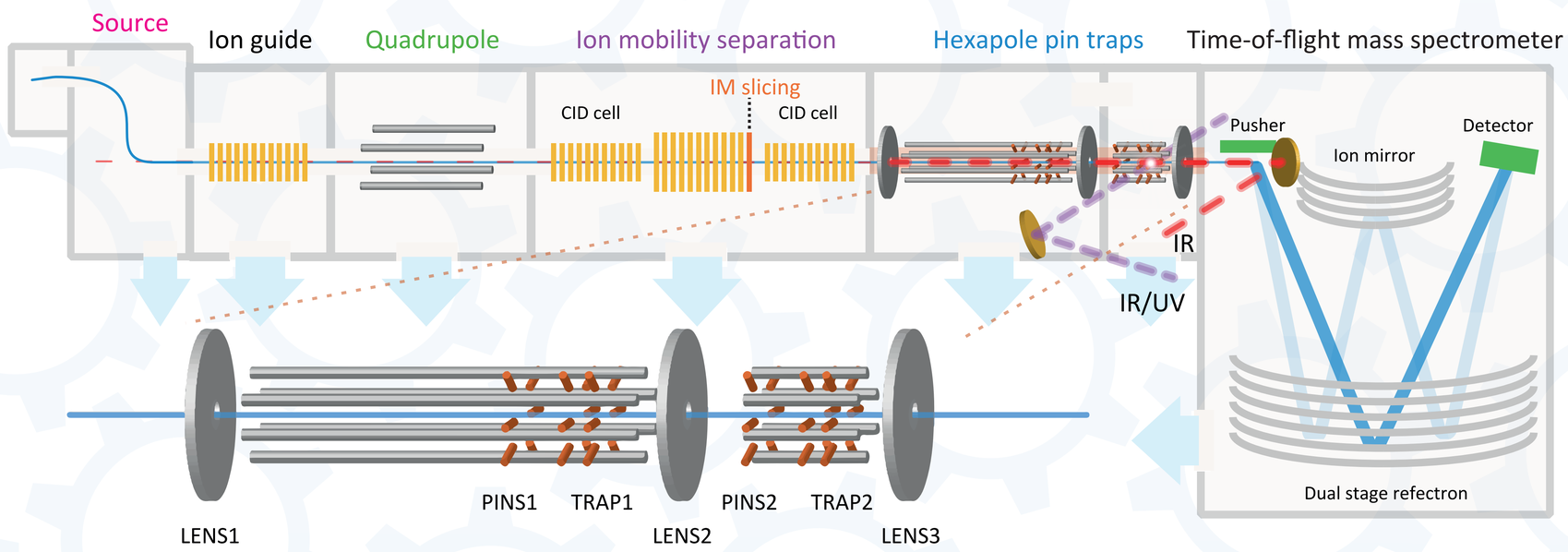


Introduction

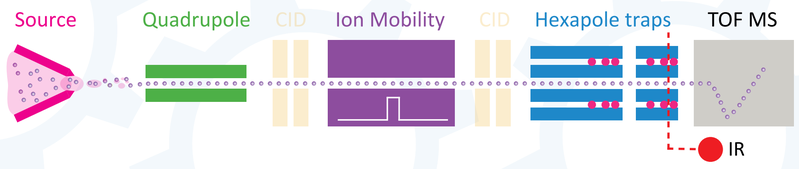
- Aggregation of peptides associated with neurodegenerative diseases such as Parkinson's and Alzheimer's Disease results in complex mixtures.
- Information about specific oligomers, thought to be the toxic species, is lacking as a result of the inability to isolate and study them.
- An ion mobility-mass spectrometer (IM-MS) was modified to allow both **mass-** and **shape** selection of the ions, after which they can get stored in an **ion trap** and irradiated by UV or IR light (Photo-Synapt).

The Photo-Synapt

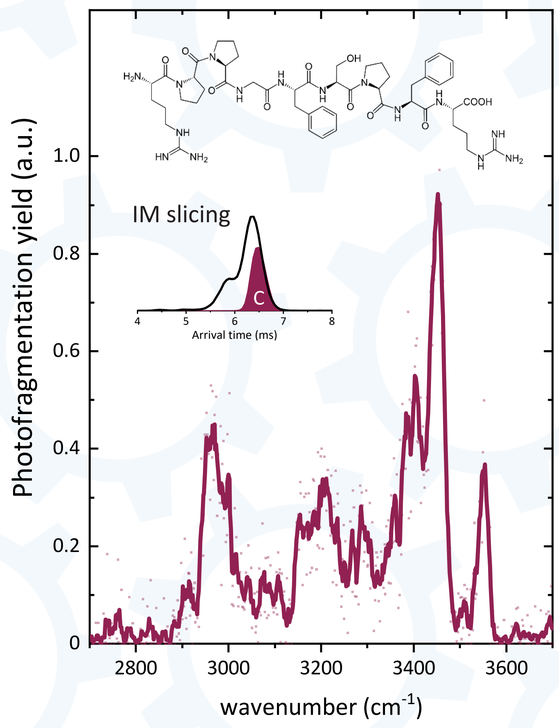
- A commercial IM-MS instrument (Waters Synapt G2) was modified by adding optical access for lasers, additional gas inlets to control the pressure in the trap, and two hexapoles pin traps.
- This design allows for m/z-selecting an ion (**quadrupole**), slicing out a specific shape or isobaric charge state (**ion mobility** slicing), trap it in the new ion **pin traps**, interrogate it with either UV or IR laser light (in TRAP2) and detect the resulting fragment masses in the mass spectrometer.
- Characterization and benchmarking experiments are currently on their way.



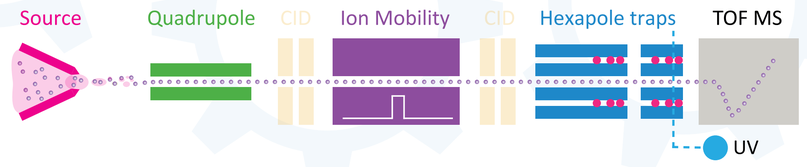
IM sliced IRMPD of bradykinin



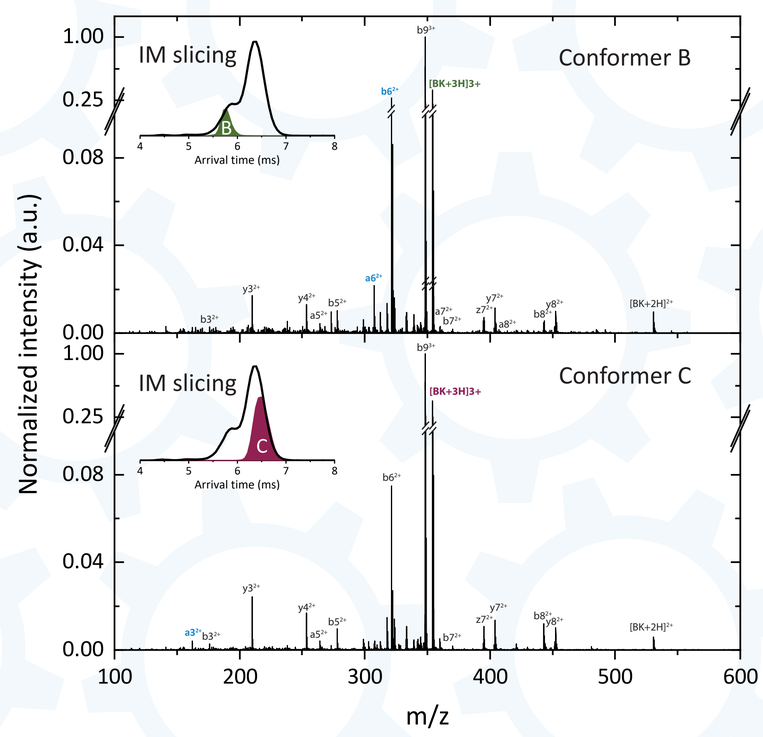
- The triply charged monomer of **bradykinin** (m/z 354, 5 µM in H_2O:MeOH, 0.1% formic acid) is a well-studied nonapeptide and can adopt **3 conformations**.
- We slice out a single conformer of this molecule using **ion mobility slicing** and trap it and irradiate for 2.5s in the hexapole ion traps to induce **IR photofragmentation**.
- The IR laser: M Squared FireFly (100 mW, 150 kHz, 2.7-4.1 µm).
- The fragmentation (via Internal Vibrational Redistribution) is monitored as a function of the wavelength.
- Fragmentation yield is calculated by $\frac{\sum \text{Fragments}}{\sum \text{Fragments} + \text{Precursor}}$

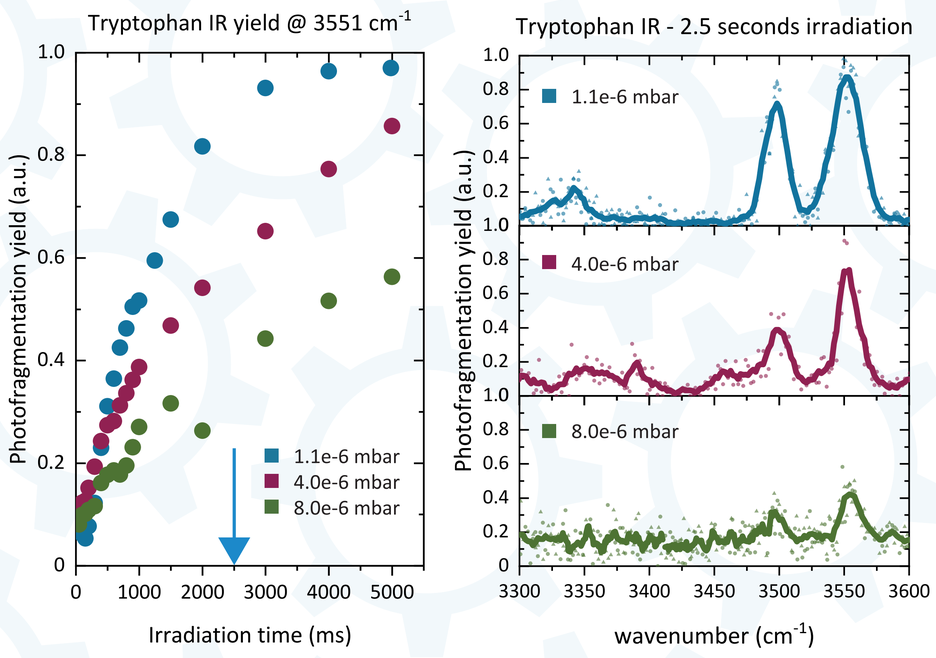


IM sliced UVPD bradykinin



- The same procedure is followed as with IRMPD (see left box): so quadrupole mass selection, ion mobility slicing and trapping and irradiating for **250 ms** of **triply charged bradykinin** in the hexapole traps at 3.1e-5 mbar.
- We irradiate the ions with a **213 nm UV laser** (CryLas eMOPA 213 (1000 Hz, 20 mW) for 150 ms.
- Both direct dissociation and internal vibrational redistribution pathways by UVPD are resulting in fragmentation, which leads to a higher sequence coverage than other fragmentation techniques such as CID.





IRMPD vs pressure

- The singly charged monomer of **tryptophan**, m/z 205, (5 µM in H_2O:MeOH) is used to characterize IRMPD on this instrument.
- The **cooling** by the nitrogen to efficiently trap the ions and the **heating** by the IR to fragment them are **competing** processes.
- Lower pressures lead to considerably higher fragmentation yields for the same irradiation time.
- The next step is to move to larger or more complex systems such as peptide aggregates.

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