

# Pushing the Boundaries of Native Mass Spectrometry: Structural Characterization of Ultra-High Molecular Weight Protein Complexes

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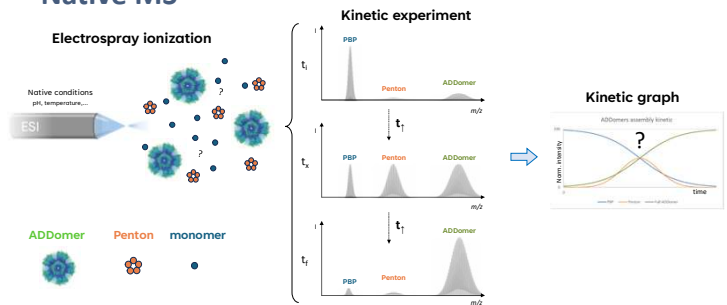


## Introduction

Ultra-high molecular weight (UHMW) proteins and their intricate non-covalent complexes play major roles across a wide spectrum of biological processes and modern therapeutic applications. From orchestrating fundamental cellular mechanisms to serving as next-generation biopharmaceuticals, these massive molecular architectures are of profound scientific and clinical interest. Deciphering the highly specific interactions within these large assemblies is essential for advancing both basic biophysics and rational drug design. The models of interest could be ranging from classical antigen-antibody complexes and the binding of neurotoxins like fasciculin to acetylcholinesterase to highly engineered synthetic platforms like ADDomers. While traditional techniques provide invaluable structural snapshots, capturing the dynamic nature, stoichiometry, and transient states of these systems remains a technical challenge. Native mass spectrometry (MS) has emerged as an innovative approach to address this, yet the routine analysis of intact species scaling from hundreds of kilodaltons up to the megadalton range requires pushing the current boundaries of instrumentation.

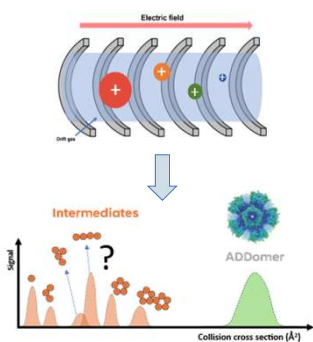


## Native MS

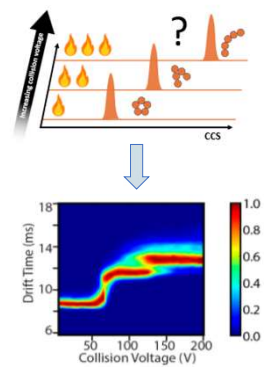


## Methods

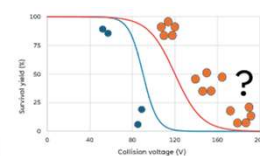
### Ion Mobility (IM-MS)



### Collision Induced Unfolding (CIU)



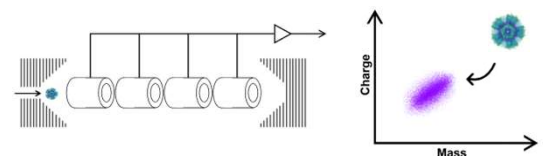
### Breakdown curve experiments



### Targeted information

- Assembly / Disassembly kinetic (IMS)
- Gas-phase compacity information (CCS)
- Intermediate discrimination (IMS)
- Conformational changes (CIU)
- Determination of the relative strength (breakdown curves)

### Charge Detection-Mass Spectrometry



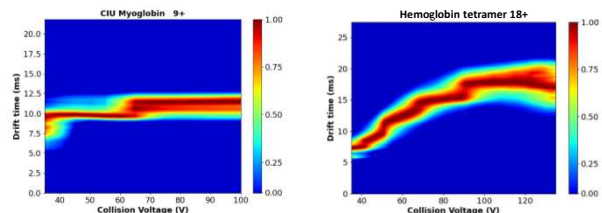
### Targeted information

- Single-particle mass analysis
- Direct mass determination in the megadalton regime
- No need for charge-state resolution
- Detection of low-abundance assembly intermediates
- Near-native characterization of intact viral assemblies



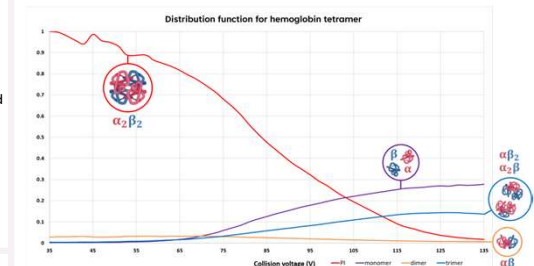
## Results

### CIU experiment on ions of increasing mass



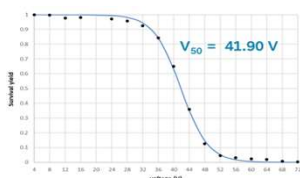
- CIU results were successfully obtained on  $[M+9H]^{9+}$  from myoglobin
- At least 3 different conformations detected
- Method extension on larger systems such as Hemoglobin
- Continuous shift of the tetramer conformation across increasing voltages

### Distribution functions

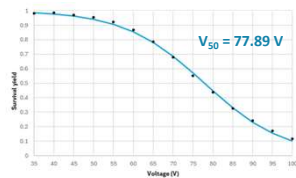


### CID experiment on ions of increasing mass

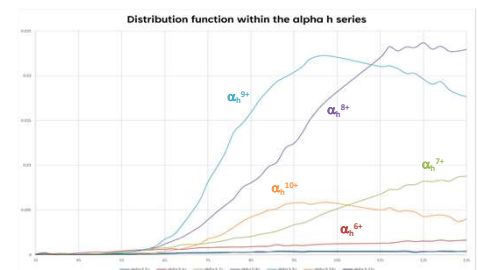
#### Breakdown curve experiment on the 5+ ion of insulin



#### Breakdown curve experiment on the 18+ ion of hemoglobin tetramer



- Breakdown curve results successfully obtained on  $[M+5H]^{5+}$  from insulin
- Well extended to increasingly larger systems
- Progressive loss of the sigmoid pattern
- The  $V_{50}$  for these charge states were successfully determined



## Take-home message & perspectives

- ✓ The project is divided into **three objectives** aiming to investigate the limits of UHMS, characterize massive supramolecular assemblies up to **ADDomers** and compare the results with innovative methods as CD-MS and finally compare gas-phase behaviors with liquid-phase analyses, elucidating limitations and areas of biophysical similarity.
- ✓ Both **native** and **non-native** conditions were tested on our systems with several **differences** observed  
Key aspect for the study of larger models (control the charge state)
- ✓ **CIU** and **CID** experiments successfully mapped **conformational stability** and **V50 breakdown** values on increasingly larger models, respectively

## What's next?

- Investigate models of **increasing mass** (conA, phosphorylase B, etc.) and complexes of biological interest using **IMS** based methods
- Implement the previously **optimized method** on the **ADDomers** and **intermediates** to compare the results with CD-MS results obtained on megadalton assemblies
- Comparison with liquid-phase methods such as DSF, BLI and SEC MALD



## Contacts



Contact and partners information