Separation of Peptide and Protein Conformers by High Resolution Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS)

Michael Groessl, Urs Rohner, Stephan Graf

TOFWERK, Switzerland. E-mail: groessl@tofwerk.com

Overview
- IMS-MS in combination with native nano-electrospray ionization can be used to investigate the tertiary and quaternary structure of peptides and proteins.
- High resolution IMS helps to identify multiple conformers.
- Low-field conditions and the ability to adjust pressure and temperature for IMS separation enable the preservation of native-like structures during analysis.
- Type and population of different conformational states can be strongly dependent on the type of solvent (protic, aprotic) used in the spraying solution.

Results
- Influence of solvent on peptide structure
- Bradykinin = Physiologically and pharmacologically active peptide, acts as an inflammatory mediator.
- Ion mobility separation was carried out at reduced field strengths of ca. 2 Td. Diffusive pressure was set between atmospheric and 1.4 atm (nitrogen).
- Measurements were carried out in both positive and negative ion mode (applied potential approx. 2 kV). Raw IMS-TOF data were post-processed using Tofware (TOFWERK, Switzerland).
- All peptides, proteins and other chemicals were obtained from Sigma-Aldrich (TOFWERK, Switzerland).
- All measurements were carried out on a TOFWERK IMS-TOF. The system comprises a nanoESI source, a 10 cm drift tube (both made from resistive glass) and a TOFWERK HTOF TOFMS.

Methods and Instrumentation
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- Desolvation and drift tube were thermostated between 30-100°C with nitrogen as the buffer gas.
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Influence of ligand binding on protein structure
- Bradykinin = Physiologically and pharmacologically active peptide, acts as an inflammatory mediator.
- Oxaliplatin (OxPt) is a clinically used chemotherapeutic.
- Binding of OxPt to ubiquitin (Ub) has already been confirmed by top-down and bottom-up proteomics. (Anal Bioanal Chem, 2012, 402:2655-2662).
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Comparison of ion mobilograms and calculated collision cross sections for holo- (with heme) and apo-forms of myoglobin for different charge states. It can be clearly seen that the collision cross sections of the apo-forms is significantly smaller compared to the apo-forms. This is in perfect agreement with data from X-ray crystallography which is a strong binding of the protein upon calcium binding. The high IMS resolution allows to resolve many substructures especially for the apo protein.

Comparison of ion mobilograms and calculated collision cross sections for holo- (without heme group) and apo-forms of myoglobin for different charge states. Under the selected conditions and charge state 8+ the holo protein is always significantly smaller compared to the apo-forms, indicating structure stabilisation by the ligand. The high IMS resolution allows to resolve many substructures especially for the apo protein.