Preserving protein nativeness in liquid chromatography and ion mobility spectrometry

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Gold statement
- Understand the purpose and challenges of native protein analysis.
- Learn that supposedly mild chromatographic conditions still may induce protein denaturation.
- Discover how TIMS can be used to study native protein conformations.

Introduction
So-called 'native' analytical techniques that permit characterization of proteins while maintaining their conformation, are essential for the study of higher order structures and structure-function relationships of proteins. Aqueous size-exclusion chromatography (SEC) at neutral pH combined with native mass spectrometry (MS) is regarded the golden standard for studying proteins in their natural state. Recently, trapped ion mobility spectrometry (TIMS) has emerged as a potential native tool for the assessment of protein conformations. Still, questions have been raised as to whether proteins actually remain native during analysis, i.e., whether the applied SEC, MS and TIMS conditions indeed prevent protein denaturation.

Body
We studied the impact of volatile mobile phases on the retention, ionization and denaturation of proteins analyzed by SEC-MS [1]. We used measured SEC distribution coefficients and protein-ion charge state distributions obtained by native MS to probe protein unfolding. This allowed monitoring of alterations in protein structure, even along SEC protein peaks. Notably, several supposedly mild eluent compositions induced nonideal SEC behavior and/or protein unfolding. From the obtained results, mobile phase compositions that do not compromise native states could be proposed.
TIMS provides unprecedented ion-mobility resolution and shows strong potential for protein structure analysis. However, standard TIMS settings are optimized for low molecular-weight compounds. Here we report TIMS conditions that (i) allow efficient trapping of high-molecular weight compounds, and (ii) preserve protein native conformation. To achieve (i), use of dopant enriched nitrogen gas for protein supercharging was considered, as well as reduction of the TIMS tunnel inlet pressure. The IM calibration was adapted to allow determination of protein CCS values. For (ii), effects of RF and DC fields in the TIMS analyzer on the protein structure were assessed and suitable values derived. Gradual increase of the DC voltage appeared effective for the *in situ* assessment of protein structure stability.

Conclusion
1. Suitable conditions for genuinely native SEC-MS of proteins were derived.
2. By selecting proper operating voltages and pressures, TIMS-MS represents an efficient new tool for the study of protein conformation.

References
[1] I.K. Ventouri, *Anal. Chem.* **92** (2020) 4292–4300.