

Subunit architecture of multiprotein complexes isolated directly from cells - Insights from mass spectrometry of intact complexes

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Introduction High throughput proteomic approaches have identified numerous *in vivo* protein complexes and suggested the existence of many others from extensive protein interaction networks. Standard proteomics techniques are however unable to describe the stoichiometry, subunit interactions and organisation of these assemblies since many are heterogeneous, present at low cellular abundance and frequently difficult to isolate.

Methods We have combined two existing methodologies to tackle these challenges: tandem affinity purification (TAP) and nanoflow ESI-MS. Our rationale is based on the selectivity offered by TAP for isolation of complexes at natural expression levels and the direct detection of complexes achievable by nanoflow ESI-MS. We use methods designed to maintain non-covalent complexes within the mass spectrometer to provide definitive evidence of interacting subunits based on the masses of complexes and subcomplexes generated by perturbation both in solution and gas phases. Ion mobility measurements are used to delineate the possible arrangements of subunits within these complexes.

Results Data will be presented for hetero-oligomeric protein complexes including the yeast exosome (1) and the human eIF3 complex (2) containing 10 and 13 different subunits respectively. These models have enabled us to propose atomic models and to gain insight into the roles of various subunits.

A very recent breakthrough has enabled us to extend the methods we have developed for soluble complexes to membrane complexes (3). By maintaining the complex within a protective micelle we have shown that we can preserve interactions between membrane and cytoplasmic subunits enabling us to define the stoichiometry and interactions in hitherto uncharacterized complexes.

Innovative aspects

- **intact membrane protein complexes**
- **complete interaction networks for novel complexes**
- **symmetry and packing of protein complexes**

References

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High resolution mass spectrometry to study functional protein complexes

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Introduction In structural biology native mass spectrometry has emerged as an excellent technique to study intact protein assemblies as the interactions present in functional complexes can be maintained in the gas-phase. With the introduction of ion mobility mass spectrometry its potential expanded even further, as now also information about the shape of the macromolecular protein complexes in the gas-phase can be revealed [1]. Here we apply high resolution mass spectrometric techniques to study the intact capsid of the human hepatitis B virus (HBV) as well as the chaperonin complex of *Escherichia coli*.

Methods

Nano-ESI mass spectra are acquired on a modified Q-ToF 1 and the ion mobility data on a Synapt HDMS, both from Waters, Manchester, UK. In the modified Q-ToF xenon was used as the collision gas. In the Synapt we used either argon or xenon in the trap and the transfer ion guides. The ion mobility cell was filled with nitrogen and a ramped wave height of 7-30 V and wave velocity of 250 m/s were used. One ion mobility separation was set at 51 ms. Drift times were corrected for mass, charge and field free region before the pusher. Calculated collision cross sections were averaged over all observed charge states.

Results The HBV capsid containing the genome forms two icosahedral geometries that consists of 180 (T3, 3 MDa) or 240 (T4, 4 MDa) copies of the capsid. In the mass spectrum these capsids structures show two distinct charge state distributions. Strikingly, both T3 and T4 capsids show two conformers in ion mobility spectra, with one conformer being approximately 4.4% larger. For the capsids the volumes determined from the cross sections (cs) are not linearly related to their masses. This clearly indicates that in the gas-phase these capsids still remain as hollow spheres.

The chaperonin complex GroEL-GroES assists in the folding of the many proteins. Upon infection of *E.coli* by bacteriophage T4 GroEL is used to fold the gp23 capsid protein. Previously we monitored all the intermediate chaperonin complexes during gp23 folding [2]. Analysis of these complexes by ion-mobility mass spectrometry revealed a clear increase in cs upon binding of the co-chaperonin to GroEL, whereas the binding of gp23 to GroEL hardly affected the cs of the complex. Evidently, the substrate is buried in the GroEL folding cavity. This clearly reveals that chaperonin complex structures can be remained in the gas-phase.

Innovative aspects

- Detailed structural and functional analysis of heterogeneous macromolecular protein complexes
- Conformational changes of intact assemblies directly monitored by ion mobility mass spectrometry

References

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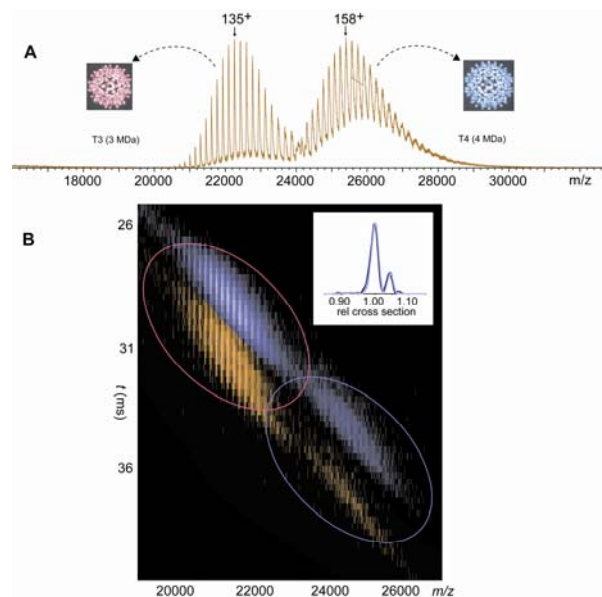


Figure 1. (A) Mass spectra of the HBV capsid shows two distributions corresponding to T3 and T4 capsids. The insets show the cryo-EM images of these capsids. (B) Ion mobility spectra of the capsids show a small (blue) and large (orange) conformer for both T3 and T4. The abundance of the conformers is depicted in the inset.

Microfluidic Platform for ESI (DESI) and MALDI (LDI) Shotgun Proteomic Analysis

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Introduction

Shotgun proteomic analyses using electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS) have been shown to observe complementary sets of peptides with incomplete overlap. For maximal proteomic coverage, it is desirable to employ both modes of analysis. We are developing a polymer-based microfluidic platform designed to interface to both ESI and MALDI MS as well as to a new dual mode ESI-DESI MS platform. These developments combined promise to increase the depth of coverage in proteomic analysis using shotgun methods.

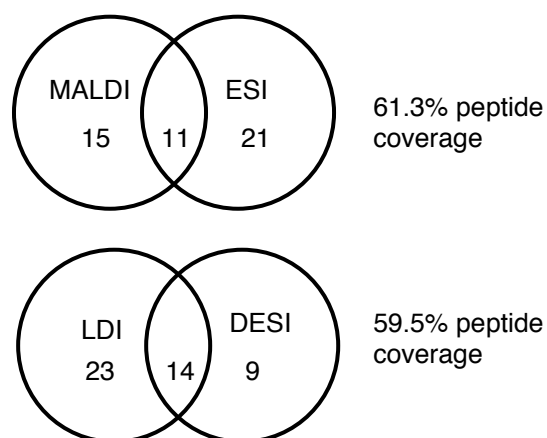
Methods

Nickel masters for microfluidic devices were prepared by double layer microlithography on nickel wafers using SU-8 photoresist and nickel plating the features. Microfluidic devices were prepared by hot embossing in cyclic olefin copolymer wafers and heat bonding cover plates. Nanoporous alumina thin films were prepared by vacuum evaporating aluminum onto glass slides and anodizing. A thin film of gold was applied to the alumina by sputtering. MALDI and LDI spectra were obtained on ABI Voyager STR and Bruker Autoflex III instruments. ESI and DESI spectra were obtained on Thermo LCQ and LTQ ion trap instruments using Thermo Nanospray ESI and in-house constructed DESI sources.

The first dimension ionization-based separation (strong cation exchange [SCX] or isoelectric focusing [IEF] is performed offline, and the fractions collected onto a microfluidic device consisting of an array of monolithic reversed phase (RP) liquid chromatography (LC) columns. The columns are eluted simultaneously in the second dimension separation, and the separation fractions collected in parallel onto a nanoporous alumina sample plate using electrostatic transfer.

Results

Analysis using LDI/DESI gave comparable results to analysis by MALDI/ESI, but with the added advantage of a single common sample format. As an example of the comparative peptide coverage for a protein digest, the following results were obtained for bovine serum albumin digest (numbers indicate numbers of peptides observed by each MS ionization method):



The LDI/DESI combination gives peptide coverage comparable to MALDI/ESI, but with the added advantage of a common sample format, thereby enabling deeper analysis of a sample spot that would otherwise be only amenable to MALDI analysis.

Innovative aspects

- Increased separation space via use of microfluidic reversed phase LC column array device.
- Increased analysis depth via use of a dual LDI/DESI ionization platform.

References

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Targeted Peptide Identification Based on Selected Reaction Monitoring

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Abstract

Proteomics shotgun strategies successfully enabled identification and quantification of proteins in complex mixtures. Typically only the most abundant proteins are analyzed due to excessive sample complexity and under-sampling of current instrumentation. Reduction of sample complexity in conjunction with selected reaction monitoring (SRM) based strategy permit quantification of peptides over a wide dynamic range with high selectivity and sensitivity [1]. The technique was further developed to systematically identify peptides with confidence by measuring multiple transitions in parallel.

Method

Proteins (isolated from yeast lysates) were digested with trypsin, and analyzed by LC-MS using C18 reversed phase column (100 μ m x 100 mm). Phosphopeptide enrichment was performed using TiO₂ chromatography, and glycopeptides were isolated with Concanavalin A and treated with Endo-H. MS analyses were performed on a triple quadrupole instrument operated in SRM mode. Typically 6-8 transitions were measured for each peptide under investigation. The instrument control software allowed for scheduled measurements. A library of transitions was built based on the fragment ions observed in MS₂ spectra of peptides already identified. Product ion spectra were measured under various collision conditions to optimize the fragmentation patterns and were stored in a library. This constitutes the resource to establish effective SRM methods.

Results

A novel SRM strategy was developed to systematically and reliably identify (confirm the identity) of peptides. It consists in measuring several SRM transitions in parallel to capture a major fraction of the ion current contained in a MS/MS spectrum, typically a dozen of fragments, represented in an abbreviated spectrum. The actual signals and their relative intensities represent specific peptide signatures. As the fragmentation patterns heavily dependent on the instrument and the collision conditions, rigorous

control of these parameters is crucial. The identity confirmation in a SRM experiment is carried out by measuring 6 to 8 transitions for each peptide, ensuring perfect co-elution of their profiles, and checking consistency with the abbreviated spectra stored in the library. The method was applied to reliably identify and quantify hundreds of peptides in one LC/MS run by effectively scheduling the SRM in narrow time windows. The method also enables detection and precise localization of phosphorylation or glycosylation sites by monitoring two series of transitions, corresponding to the modified and unmodified fragments. This demonstrates the power of parallel SRM analyses to identify and characterize peptides in complex mixtures with unprecedented sensitivity.

Innovative aspects

- Parallel SRM analysis of peptides in complex mixtures, with unprecedented sensitivity.
- Concomitant identification and quantification of hundreds of peptides by SRM, using scheduled measurements.
- Application of SRM to detect and localize post-translational modifications, including glycosylation.

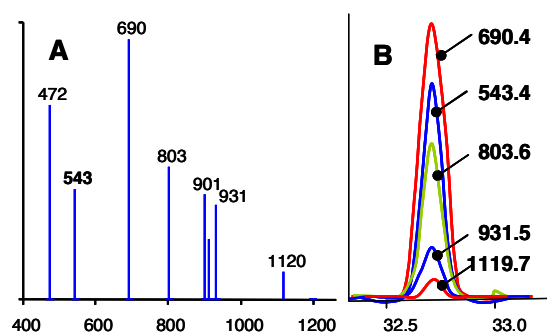


Figure: A) Abbreviated MS/MS spectrum of the peptide QAIECLEGMQLFAPEVK (3+ precursor); B) Major SRM transitions.

Reference

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