Native/Supramolecular Mass Spectrometry to Decipher Interactions between Biomolecules: past, present and future.

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Supramolecular chemistry refers to chemical systems made up of a number of assembled molecular subunits.

The forces responsible for the spatial organization may vary from
- weak (intermolecular forces, electrostatic or hydrogen bonding) to
- strong (covalent bonding)

The Nobel Prize in Chemistry 1987 was awarded jointly to
- Donald J. Cram,
- Jean-Marie Lehn and
- Charles J. Pedersen
"for their development and use of molecules with structure-specific interactions of high selectivity".

Supramolecular MS started with chemistry
Supramolecular MS started with chemistry

- FAB-MS and even MALDI-MS were used successfully since 1985 for the characterization of many coordination compounds such as Cu+/ bipyrene based pentahelicates

- ESI-MS was used from 1989:
  - to determine the stoichiometry of supramolecules
  - to determine their assembly pathways
  - to determine their kinetic and thermodynamic constants

- These studies showed that key parameters were mainly:
  - the control of desolvation in the ESI interface,
  - the homogeneous transmission of heavy ions and
  - the importance of control experiments.

For biological molecular assemblies these three points are also of major importance, but much more difficult to address than for synthetic supramolecules
In 1991, two pioneering groups reported the detection of noncovalent complexes by MS.

Observation of the Heme–Globin Complex in Native Myoglobin by Electrospray-Ionization Mass Spectrometry

Viswanatham Katta and Brian T. Chait*

*The Rockefeller University, New York, New York 10021
Received May 8, 1991


Detection of Noncovalent Receptor–Ligand Complexes by Mass Spectrometry

Bruce Ganem*

Department of Chemistry, Baker Laboratory
Cornell University, Ithaca, New York 14853

Yu-Tsyr Li and Jack D. Henion*

Drug Testing and Toxicology
New York State College of Veterinary Medicine
Cornell University, 925 Warren Drive
Ithaca, New York 14850
Received April 24, 1991


ESI is soft enough to transfer non-covalent complexes from the solution into the gas phase of a mass spectrometer.
Technological breakthroughs in MS allowed detection of assemblies of always higher molecular weights.

ESI-MS of biomolecules (J. B. Fenn)  1988

nanoESI (M. Mann)  1996

Automated nanoESI-MS (Zhang + Henion)  2000

Improvements in TOF and Q-TOF technologies

Ion Mobility - Mass Spectrometry (IM-MS, Synapt HDMS)  2006

First ESI-MS mass spectra of noncovalent complexes (Chait + Henion)  1991

nanoESI needles for noncovalent complex analysis (Robinson CV group : transthyretin)  1998

Automated nano ESI for native MS (Robinson CV group : transthyretin)  2003

Native MS it is now widely used to study noncovalent complexes in structural biology  2012

From the beginning, a central question was raised:
Do ESI mass spectra faithfully reflect the equilibrium that preexists in solution?
Can valuable information be deduced from native mass spectra?

- Can binding **stoichiometry** information be deduced?
- Can we measure **binding constants** to have solution affinities?
- **How large** can we measure? Is it possible to measure several millions of Daltons?
- Is it possible to **monitor kinetics of assembly/disassembly**?
- Is the **conformation** of a protein/complex maintained in the gas phase?

Today, native MS is an established technique for non-covalent complex characterization, especially in terms of binding stoichiometries.
Native MS for binding stoichiometries determination
Ex: protein/small molecule system

Determination of a 1:1 protein/ligand stoichiometry
Specific binding of one actinonin molecule to one PDF1β

No nonspecific binding

PDF1β
(5 µM)

+ 1 eq. actinonin
(5 µM)

+ 3 eq. actinonin
(15 µM)

21934 ± 1 Da

22320 ± 1 Da

+ 386 Da

m/z

2440 2470 2500 2530

21934 ± 1 Da

22320 ± 1 Da

No nonspecific binding

Determination of a 1:1 protein/ligand stoichiometry
Specific binding of one actinonin molecule to one PDF1β
Native MS for binding stoichiometries determination
Ex: determination of the oligomeric state of multimeric enzymes

HPrK/P a bifunctional enzyme: \( \text{MW}_{\text{mes}} = 51,700.0 \pm 1.0 \text{ Da} \)

\[310,337 \pm 22 \text{ Da}\]

- **pH = 6.8**
  - Phosphatase Activity (dephosphorylation)
  - HEXAMER

- **pH = 8.5**
  - Kinase Activity (phosphorylation)
  - DIMER - TRIMER

Relation between structure and activity

Ramstrom *et al.*, *J. Biol. Chem.*, 2003, 14, 419
Native MS for binding stoichiometries determination
Probing Cooperative Cofactor Binding on Multimeric Enzymes

ADH (20 µM in tetramer)

GPDH from sturgeon muscle (20 µM in tetramer)

No Cooperative Binding

Strong Cooperative Binding

Native MS for binding stoichiometries determination

Ex : protein/protein interactions

mAb (5 µM)

mAb (5 µM) + JAM-A (10 µM)

mAb (5 µM) + JAM-A (40 µM)

mAb:Ag 1:4 stoichiometry

Unexpected binding stoichiometry for a mAb/Ag system!

Atmanene et al., Anal. Chem. 2009, 81, 6364
Determination of CggR/DNA binding stoichiometries:

one tetrameric CggR binds to one DNA molecule

CggR (5 µM in tetramer)

CggR + DNA (3 µM in complex)

4/1 CggR/DNA complex

Measured MW
155145 ± 3 Da*

Measured MW
182868 ± 9 Da

Atmanene et al., Anal. Chem. 2010, 82, 3597
Can native MS serve to determine solution binding affinities?

Loss of hydrophobic interactions during transfer into the gas phase.
Can native MS serve to determine solution binding affinities?

**Vc50 measurements** to determine the type of interaction involved in complex formation:

Gas phase stabilities are correlated to the type of interactions involved in complex formation: gas phase stabilities reflect polar contribution of the interaction.

Rogniaux *et al.* (1999) JASMS, 10, 635-647
Can native MS serve to determine solution binding affinities?

For complexes maintained by polar interactions, solution affinities can be deduced from gas phase native MS measurements.

Native MS for hit/lead validation in drug discovery processes

All steps of the process can be automated: protein/ligand mixtures, nanoESI injection, MS acquisition, data interpretation.

Sample preparation and nanoESI automation

Automated ESI-TOF MS acquisition

Stoichiometries? Affinities?

Intensity

Da

26500 27000 27500

For all experiments, interacting partners were incubated at 10 µM each.
Native MS for \textit{in vitro} reconstruction of non-covalent assemblies

\textbf{Guide RNA}

\begin{itemize}
  \item $18903 \pm 1 \text{ Da}$
  \item $78064 \pm 1 \text{ Da}$
\end{itemize}

\textbf{ACA} $+ \text{L7Ae}$

\begin{itemize}
  \item $32326 \pm 1 \text{ Da}$
  \item $82254 \pm 7 \text{ Da}$
\end{itemize}

\textbf{ACA} $+ \text{Cbf5/NOP10}$

\begin{itemize}
  \item $81000 \text{ M (Da)}$
\end{itemize}

\textbf{ACA} $+ \text{Substrate RNA}$

\begin{itemize}
  \item $84000 \text{ M (Da)}$
\end{itemize}

\textit{in vitro} reconstruction of the active H/ACA RNP particle
Native MS to probe dynamics of assembly/disassembly

Affinity tagged RNA Pol I

Lane et al. Structure 2011, 19, 90-100
How large can we measure today?

Native MS

Holo-myoglobin
17.5 kDa
1991

Haemocyanins
2.3 MDa
2002

Viral capsids
4 MDa
2008

Norwalk Virus
10.1 MDa
2012

1998
GroEL
800 kDa

2006
Ribosomes 70S
2.3 MDa

Robinson CV et al.

McKay et al. JACS 2006

Sanglier et al. JASMS 2002

Katta et al. JACS 1991

Uetrecht et al. PNAS 2008

Shoemaker et al. MCP 2010
Future challenges: for high MW measurements...

Issues are more related to the heterogeneity of protein assemblies than to MS capabilities to measure high MW.

30S = 21 proteins (~300 kDa) + 16S RNA (~500 kDa)  
50S = 34 proteins (~450 kDa) + 5S RNA (~40 kDa) + 23S RNA (~1 MDa)
Is the native conformation of a biomolecule maintained throughout the ionization and analysis process?

- 1996: 1st proof that active proteins can survive into a mass spectrometer are given by Siuzdak G., et al. Chemistry & Biology, 1996, p.45

- Since then, several groups reported strong hints that protein conformations are retained within a mass spectrometer

- 2012: The overall structure of individual proteins and protein complexes in buffered solutions is not generally influenced by charge state

Hall and Robinson (2012) JASMS, 23, 1161-1168
Simultaneous measurement of drift times and m/z ratios

Combining Ion mobility and native MS to gain information on protein/complex conformations

ESI source → IM cell → MS analyzer → Detector

Drift time

Time of flight

Simultaneous measurement of drift times and m/z ratios
Combining Ion mobility and native MS to gain information on protein/complex conformations

Ion separation takes place according to ion mobility

- Drift times can be related to collisional cross sections (CCS)
- Information on ion gas phase conformation

Charge
- Size, shape
- Compactness
- Drift time

Ions separation takes place according to ion mobility

Drift time

Combining Ion mobility and native MS to gain information on protein/complex conformations
IM-MS to detect subtle conformational changes on protein/ligand systems

Detection of subtle conformational changes:
~ 2% difference between CCS of apo and holo forms (<1nm²)

CCS_{apo} = 16.7 ± 0.2 nm²
CCS_{holo} = 17.2 ± 0.3 nm²

ΔCCS = 0.5 nm²

**Problem**: CggR is a tetrameric protein that interact with its specific DNA (183 kDa)

**Question**: Is there a conformational change induced by FBP binding (Fructose bis-phosphate) ?

**Hypothesis**: IM-MS to detect conformational changes induced upon small molecule binding on large non-covalent assemblies
IM-MS to detect conformational changes induced upon small molecule binding on large non-covalent assemblies

- CggR/DNA 3 µM + 30 µM FBP

CCS (without FBP) = 9114 Å²
CCS (with FBP) = 9566 Å²
Δ CCS = + 5% upon FBP binding

Atmanene et al., Anal. Chem. 2010, 82, 3597
Future challenges: using noncovalent MS approaches to have structural informations

Native IM-MS data acquisition

Experimental CCS determination

<table>
<thead>
<tr>
<th>IM-MS</th>
<th>Mean</th>
<th>CCS (Å²)</th>
<th>9168</th>
</tr>
</thead>
</table>

Building of model of individual subunits

Theoretical CCS coming from X-ray, NMR, molecular modeling, etc...

EHSS 10213 Å²
PA 7874 Å²

Building of a 3D model structure
From IM-MS data to model structures

P domain of norovirus capsid protein

From IM-MS data to model structures

P domain of norovirus capsid protein

Linear relationship between CCS and MW is observed

⇒ Similar geometry structures

From IM-MS data to model structures:
Integration of native MS and IM-MS data to generate topological models of protein complexes

Mass Spectrometry for large noncovalent complex analysis

The next frontiers in proteome research is the study of protein-ligand interactions, protein-peptide and protein-protein complexes, and entire protein interaction networks by high-throughput methods.
Native MS: realistic perspectives? We can analyse the proteome of a cell, why not thinking about the complexome?

SM structurale:
Apport d'informations tridimensionnelles de molécules et de complexes

Kühner et al. Science 2009, 326, 1235
Many thanks for your attention!

«The native MS» team at the LSMBO
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Alain Van Dorsselaer

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To our COLLABORATORS