Bioanalytical Instrumentation --
Mass Spectrometry

Lecture 18: MS in Drug Discovery and Pharmacokinetics

Lingjun Li, University of Wisconsin-Madison
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Tsinghua University, Beijing, China

What is Drug Discovery?

The process of creating a chemical compound with chemical, biological, pharmaceutical, pharmacokinetic and toxicological properties suitable for development as a useful clinical therapeutic agent.

**CHEMICAL:** Practical for large-scale synthesis; not reactive

**BIOLOGICAL:** High affinity, selective ligand for a designated biological target

**PHARMACEUTICAL:** Soluble, stable, crystalline

**PHARMACOKINETIC:** Orally absorbable; distributes to the target organ; adequate persistence in the body; eliminated in a reasonable amount of time

**TOXICOLOGICAL:** Adequate margin of safety with respect to general toxicity and carcinogenicity
Presently Known Drug Targets

- Enzymes (47%)
- G protein-coupled receptors (30%)
- Ion channels (7%)

A biological molecule usually becomes a drug target b/c a disease is prompted by its activity, and this can be moderated by a drug.

Proteomics and Drug Discovery

- Sequencing the human genome is only the first step toward a deeper understanding of human biology and diseases and provide new avenues for drug discovery.

- Understanding the human genome is important, but most pharmaceuticals target proteins. The study of proteins and their functions might bridge the gap between drug discovery and human genomic information.

- With the structure of proteins and computer software, one may find new drugs to inactivate proteins involved in disease.

- Understanding the proteome, the structure and function of each protein, and the complexities of protein-protein interactions will be critical for developing effective diagnostic techniques and disease treatments.

Comparison of activity-based probe profiling (ABPP) and compound-centric chemical proteomics (CCCP)

- ABPP requires the formation of covalent bond between the probe molecule and the protein

- For CCCP approach, the compound of interest is conjugated chemically to an insert and biocompatible matrix in a way that does not interfere with its activity

Novel Drug Target Discovery by Proteomics

2D gel electrophoresis revealed proteins with altered mobility on treatment of cells with the cytostatic agent bengamide. MS identification indicate that the differences resulted from altered amino-terminal modification, suggesting that the target of bengamide is Met aminopeptidase.


MALDI-MS identification of an unprocessed 14-3-3γ isoform

Lysates of H1299 cells treated with LAF389 (synthetic analogue of bengamide)

Trypsin digestion

Lys-C digestion

**Table 1. Major components of the discovery phase**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Numbers of compounds</th>
<th>Role or opportunity for MS-based methods</th>
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<tbody>
<tr>
<td>Initial lead discovery</td>
<td>Start with ~10^5; prefer 10-100 hits</td>
<td>Limited by massive installed base of other methods. Current paradigm requires only single-point estimate of activity, because low-potency hits are expected (therefore, power of MS-based systems may overwhelm the task). Mass-based recognition of compounds may be thwarted by isobaric compounds or impurities</td>
</tr>
<tr>
<td>Lead optimization</td>
<td>Start with 1-4 hits; expand to 100-1000 by library technology</td>
<td>May be optimum location for use of MS; at this stage, there is interest in accurately determining the respective affinities of compounds derived from the initial leads. Requires screening shift to a secondary assay that could introduce a lag time following early screening phase.</td>
</tr>
<tr>
<td>Candidate selection</td>
<td>From a small set of advanced leads, serial synthesis is used to identify final candidate</td>
<td>Limited, as complex mixtures or large numbers of compounds are no longer being assayed; more traditional pharmaceutical methods can be applied. Use of MS methods introduced in earlier phases may continue.</td>
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</table>

**MS-based methods proposed for use in lead discovery**

<table>
<thead>
<tr>
<th>Method</th>
<th>Target</th>
<th>Potential leads</th>
<th>Principle</th>
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<tbody>
<tr>
<td>Frontal-affinity chromatography-MS</td>
<td>Immobilized in a column</td>
<td>Pumped through column</td>
<td>Compounds in dynamic equilibrium with immobilized target. Unbound and weakly bound compounds eluted earlier than bound. Mass-specificity detection</td>
</tr>
<tr>
<td>Pulsed ultrafiltration-MS</td>
<td>Mixed w/ multiple compounds in solution</td>
<td>Mixed w/ target in solution</td>
<td>Target mixed w/ potential ligands is placed over ultrafiltration membrane; when pressure is applied, ligands showing affinity for the protein are selectively concentrated; later, they are identified by MS</td>
</tr>
<tr>
<td>Affinity size-exclusion-MS</td>
<td>Mixed w/ multiple compounds in solution</td>
<td>Mixed w/ target in solution</td>
<td>Rapid molecular exclusion fractionation in a spin column separates target-ligand complexes from unbound compounds; MS identifies binders</td>
</tr>
<tr>
<td>Ultrafiltration-MS</td>
<td>Mixed w/ multiple compounds in solution</td>
<td>Mixed w/ target in solution</td>
<td>Target mixed w/ ligands and subjected to centrifugal ultrafiltration; binding compounds separated from non-binders that are washed to waste; ligands bound to target are eluted by acidification and detected by MS</td>
</tr>
<tr>
<td>Affinity capillary electrophoresis-MS</td>
<td>In electrophoretic buffer</td>
<td>In running buffer for CE</td>
<td>Bound ligands measured by mobility change of ligand upon interaction w/ target in electrophoretic buffer and identified by MS</td>
</tr>
<tr>
<td>SPR-MS</td>
<td>Coupled to optical sensor surface</td>
<td>Flow across sensor surface</td>
<td>Change in surface refractive index to detect presence of a binding partner for an immobilized target; MS identifies the binding partner</td>
</tr>
<tr>
<td>Affinity capture-MS</td>
<td>Immobilized on beads</td>
<td>Incubated w/ immobilized target</td>
<td>Bead-bound target mixed w/ potential ligands; unbound ligands removed by washing; bound ligands eluted and identified by ESI-MS/MS</td>
</tr>
<tr>
<td>Noncovalent affinity-MS</td>
<td>In gas phase</td>
<td>Mixed w/ one or multiple targets</td>
<td>Direct mass analysis of target-ligand mixture; complex of a ligand w/ a given target (or multiple targets) is identified directly from its mass using very soft ionization from volatile buffer</td>
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</table>
A: Pulsed ultrafiltration-mass spectrometry (PUF-MS) used as screening method. The target macromolecule is retained by a porous membrane which allows low-mass compounds to pass freely. B: MS-based detection of a single component in a library with affinity for adenosine deaminase.

Frontal affinity chromatography-MS (FAC-MS). The target for soluble small ligands is immobilized in a column, and a mixture of potential ligands is pumped through at a known flow rate with an in-line mass spectrometer serving as the detector. Panel A: Three stages of an experiment in which a mixture of three components is analyzed. Panel B: Elution profiles of the analytes, which are distinguished from each other by mass analysis. Panel C: An example of FAC-MS data illustrating normalized total ion count elution curves. Panel D: Representative individual mass spectra at differing time intervals.
A Mixture-Based Combinatorial Library Strategy: High-Throughput SEC Coupled to MS for Lead Discovery

Lead Optimization

- HTS provides compounds with desired biochemical activity, reducing one million compounds to 10-100 hits
- Increasing potency, pharmacokinetic properties and safety issues need to be considered during lead optimization phase
- Optimization of lead compounds carries a project to the point at which a drug candidate is nominated into the development process
  -- Lead differentiation and structure-activity relationships
  -- Fragment assembly approaches
Applications in Combinatorial Chemistry

• Combinatorial chemistry is used to create large populations of molecules or libraries.

• Drug development has been significantly facilitated by the use of the combinatorial libraries with the aid of mass spectrometry.

• An efficient qualitative and quantitative assay is crucial to distinguish the most active component or obtain structure-activity relationship of compounds in a library.

• Both ESI and MALDI MS have been useful for high throughput screening of combinatorial libraries.

• No need for chromophore or radiolabeling
(A) Experimentally measured mass spectrum of a 400-component library. (B) Predicted mass spectrum of the same library synthesized by split-couple-recombine methodology. A statistical comparison of the two spectra was made, showing a 0.81-0.82 correlation (a correlation value of 1.00 corresponds to an exact match between predicted and measured spectra).


Monitoring Enzyme Inhibition with MS

Siuzdak, 2003
Optimizing Cyclosporin A Extraction from Blood

- A combinatorial extraction method was used with automated MALDI MS to improve quantitative clinical analysis of the immunosuppressant drug cyclosporin A from blood.
- Extraction optimization was performed by generating an array of solvent systems.
- The first generation of experiments revealed four useful solvent systems, and the second iteration of experiments produced an effective 70:30 hexane/CHCl3 extraction solvent system.

Potential Applications of ESI-MS in Drug Discovery

Application of ESI-FTICR MS in ligand screening

7 μM carbonic anhydrase (CAH) holoprotein in the presence of benzenesulphonamide inhibitors w/ different AA


Table 1: Mass spectrometry techniques used in the drug discovery process

<table>
<thead>
<tr>
<th>Technique</th>
<th>Metabolite</th>
<th>Application</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Multitarget affinity/specificity</td>
<td>Direct mass spectrometric screening of multiple target complexes</td>
<td>High throughput screening of drug discovery</td>
<td>Can identify low-affinity components missed by traditional screens</td>
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<tr>
<td>Mass spectrometry (MS)</td>
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<tr>
<td>Structure-activity relationship</td>
<td>Determination of complex stoichiometry and dissociation constants</td>
<td>High-throughput affinity screening</td>
<td>Rapid screening of complex ligands</td>
<td>Production of information about binding affinities and selectivity</td>
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<td>(SAR)</td>
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<tr>
<td>Dynamic light scattering</td>
<td>Differentiation between bound and free ligands</td>
<td>Diffraction-free detection of complex interactions</td>
<td>Microwave-dissociation MMMS, provides more information than NMR</td>
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<tr>
<td>Mass spectrometry (MS)</td>
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<td>Nuclear spin relaxation spectroscopy</td>
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<td>Dissociative constant (K_a)</td>
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<tr>
<td>Determination of complex stoichiometry and dissociation constants</td>
<td>High-throughput screening</td>
<td>Diffraction-free detection of complex interactions</td>
<td>Microwave-dissociation MMMS, provides more information than NMR</td>
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<td>CAD (calculation of dissociation</td>
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<td>Constant)</td>
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<td>Constraint-based dissociation</td>
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<td>mass spectrometry (CAD-MMMS)</td>
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<td>Isoelectric focusing mass</td>
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<td>spectrometry (IEF-MS)</td>
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<tr>
<td>Ladder analysis</td>
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<td>Immunoaffinity mass spectrometry</td>
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<td>(IMM-Ms)</td>
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<td>Chromatography</td>
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Application of ESI-MS in determining binding modes

**Multitarget Affinity/Specificity Screening (MASS)**

- Determine exact chemical composition of ligands that bind to an RNA target
- Determine relative or absolute dissociation constants (K_D) of ligands for the RNA target(s)
- Determine the location of ligand binding on the RNA
- Determine the specificity for one RNA relative to 3-5 different RNA targets
- Use standard 96-well plates w/ robotic liquid handler.
- MASS assays can be run w/ solutions containing magnesium and 200 mM ammonium acetate buffer to aid RNA folding, and 2% DMSO to dissolve potential ligands.
- Screen up to 100 compounds against 5 RNA targets simultaneously in 1 minute
Multitarget Affinity/Specificity Screening (MASS)

High Precision Mass Determinations

High Precision ESI-FTICR Mass Measurement of 16S A site RNA/Paromomycin Complex

Δ m/z = 123.0094
Δ MW = 612.2666 (+ 0.00006)

M+M = 100,000 (FWHM)


http://www.ibisrna.com
Characterization of Non-Covalent Complexes

Plot of (Complexed 16S)/(Free 16S) vs. aminoglycoside concentration. $K_d$ was determined from the inverse of the slope for the plot.

MASS of 16S and 18S RNAs with a Library of Six Aminoglycosides

Griffey et al., PNAS 96, 10129 (1999).
Gas Phase Dissociation of RNA-Ligand Complexes

Probing Molecular Structure with FTICR-MS and IRMPD

Infrared laser is a "tunable nuclease"

- Increase Internal Ion Energy
- Desolvate "wet" ions
- Conformation-dependent nuclease
- Sequence, structure-independent nuclease
- Cleave most C-O and C-N bonds

Using IRMPD MS/MS to Map 16S Binding Epitopes for Paromomycin and Ribostamycin

(Ratio RNA Fragmentation in Complex Compared to Free RNA)

* = Amino group in direct contact with 3'-phosphate or base in NMR structure


Applications in Pharmacokinetics

- Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion (ADME) of drugs.
- LC coupled with soft ESI or APCI with triple quadrupole mass analysis is the primary tool in pharmacokinetic studies.

Metabolite Identification and Quantitation

- Used for MS Short Course at Tsinghua
  by R. Graham Cooks, Hao Chen, Zheng Ouyang, Andy Tao, Yu Xia and Lingjun Li
To investigate the pharmacokinetics of caffeine in 7 rats, plasma levels were measured over 24 hours. The gradual clearance of the drug could be observed over time.


**The Need for High-Throughput ADME Assays in Drug Discovery**

- Large number of hits are now routinely identified from screening compound collections and gene family compound libraries.

- 2/3 of compounds entering clinical development fail due to poor ADME properties or efficacy.

- The paradigm shift of drug metabolism and pharmacokinetic (DMPK) profiling from its late stage position to early drug discovery.

- The wide-spread incorporation of LC/MS and LC/MS/MS enables high throughput ADME assays.
Cassette Dosing

**Principle:** If several compounds are administered to an animal simultaneously, and LC-MS/MS allows us to measure each one in the same plasma sample without interference, then the slow dosing and sample collection phase is minimized.

**Advantages:** Dosing and sample collection time much shorter. Animal usage reduced.

**Disadvantages:** Risk of drug-drug interactions that will distort the results.

Very popular in the pharmaceutical industry, especially among medicinal chemistry groups.

The Cassette-Accelerated Rapid Rat Screen (CARRS)

- Use 96-well plate technology to facilitate automation of sample preparation, injection into LC-MS, tracking of data.
- Assay compounds as “cassettes” of 6 similar compounds to minimize number of mobile phase changes and maximize ease of data collection.
- Use overnight runs so that instrument is fully utilized.

Semi-Automated Sample Preparation

- Pipette 50 µl plasma (pooled from two rats) in a 96-well plate.
- Add 150 µl acetonitrile + internal standard (structural analog) using Tomtec Quadra 96.
- Vortex
- Centrifuge
- Transfer supernatant to 96-well plate using Tomtec Quadra 96.

Mass chromatograms from a typical analysis of one compound. Showing the total ion chromatogram (TIC) and the mass chromatograms for the dosed compound (SCH 12345), the internal standard and two +16 metabolites (A and B) that were also detected.

Example report format in Excel® showing the sample concentrations, the calculated AUC (area under the curve) and the time vs. concentration plot for the dosed compound.

Korfmacher et al., RCM, 15, 335-340 (2001).

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Semi-automated sample preparation procedure using 96-well plate technology.

Korfmacher et al., RCM, 15, 335-340 (2001).
IMS Analysis of PEP-19 in Brain Tissue Sections of an Animal Model of Parkinson’s Disease

Skold et al., JPR 2006

Reyzer et al., JMS, 2003, 38, 1081-1092.
CAD of standards run on a Pulsar QqTOF.
CAD of (A) the sinapinic acid cluster [3 × SA + Na+] and (B) [M + H]+ of SCH 226374. Inset is a close-up of the fragment ion at m/z 228.1, showing the presence of distinctive 13C and 37Cl isotope signals.
Image of SCH 226374 in mouse tumor tissue. The mouse was dosed at 80 mg/kg and the tumor was excised 7 hr after the last dose. (A) Optical image of tissue section (after coating with sinapinic acid). (B) Mass spectral image of SCH 226374 in the tumor section, utilizing selected reaction monitoring of the transition 695->228. White indicates no signal, black indicates high signal. The drug appears to be localized in the periphery of the tumor section.
Summary

• MS plays an important role in all aspects of drug discovery and drug development, including high-throughput ADME profiling.

• The MASS approach allows large numbers of compounds to be screened against multiple RNA targets simultaneously, resulting in greatly enhanced sample throughput and information content.

• Parallel separations and parallel analysis will be increasingly implemented in pharmaceutical industry for increased throughput.

• Quantitative proteomics provide powerful tool for drug target discovery and disease pathway profiling.

• MALDI-based tissue imaging shows potential for biomarker discovery and drug distribution profiling.