

METABOLOMICS

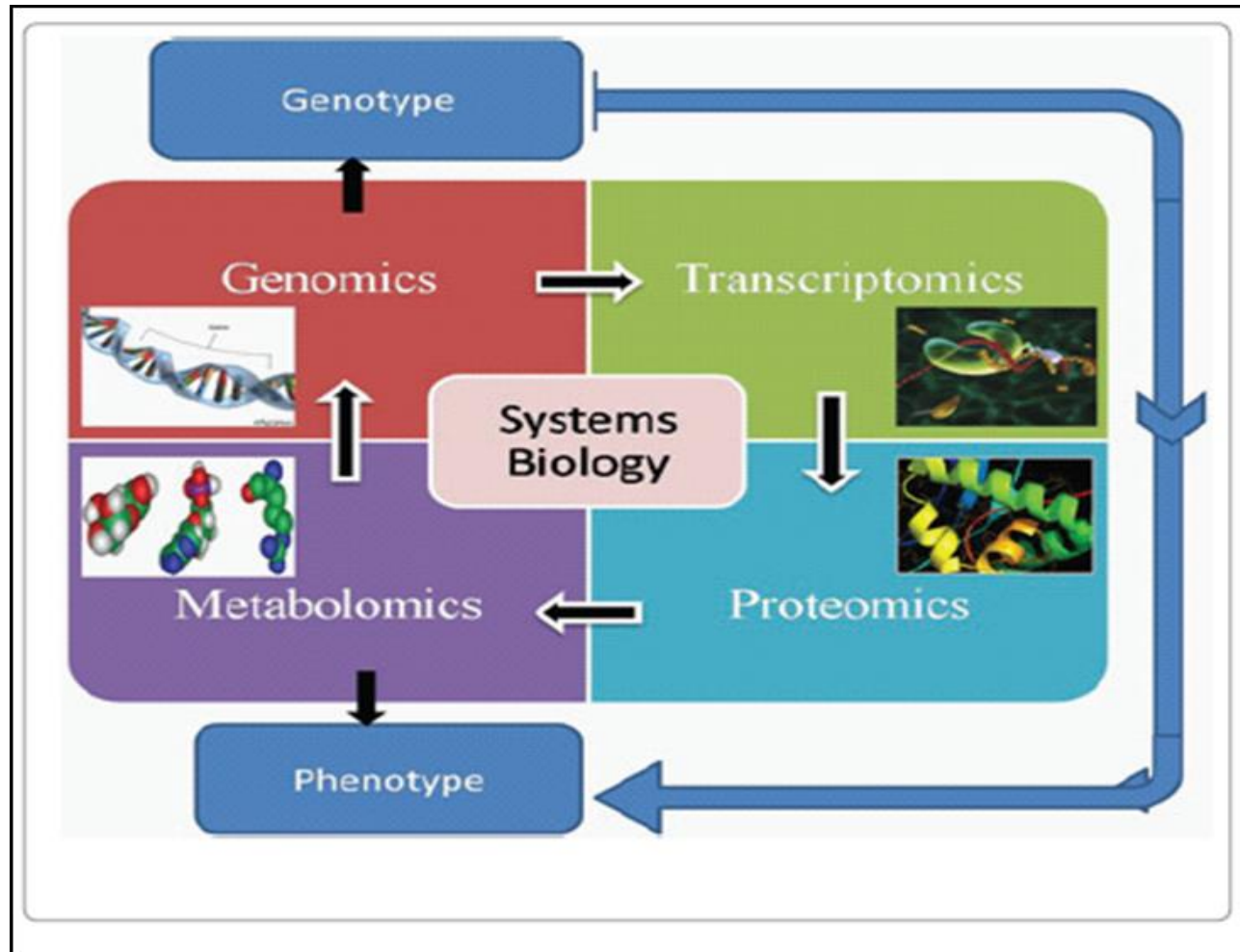
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What is Metabolomics ?

- Metabolomics is a scientific approach or discipline that aims at the comprehensive identification and quantitation of all cellular metabolites within a biological system.

Metabolomics in relation to the other 'omics' technologies



Definitions and concepts used in Metabolomics

LC-MS, CE-MS,
GC-MS, GCxGC-MS,
LC-NMR-MS
MS, NMR

Target Analysis

Single class of compounds
(ignoring all non target compounds)

Metabolite Profiling

High complexity
(Multiple classes of compounds)

LC-MS, GCxGC-MS,
ICR-FT/MS, NMR

Metabolic fingerprinting/footprinting

Very complex profiles
(multiple profiles of compounds)

Metabolomics

Identification and quantification of
all metabolites (whole metabolome)

Primary vs. secondary plant metabolites

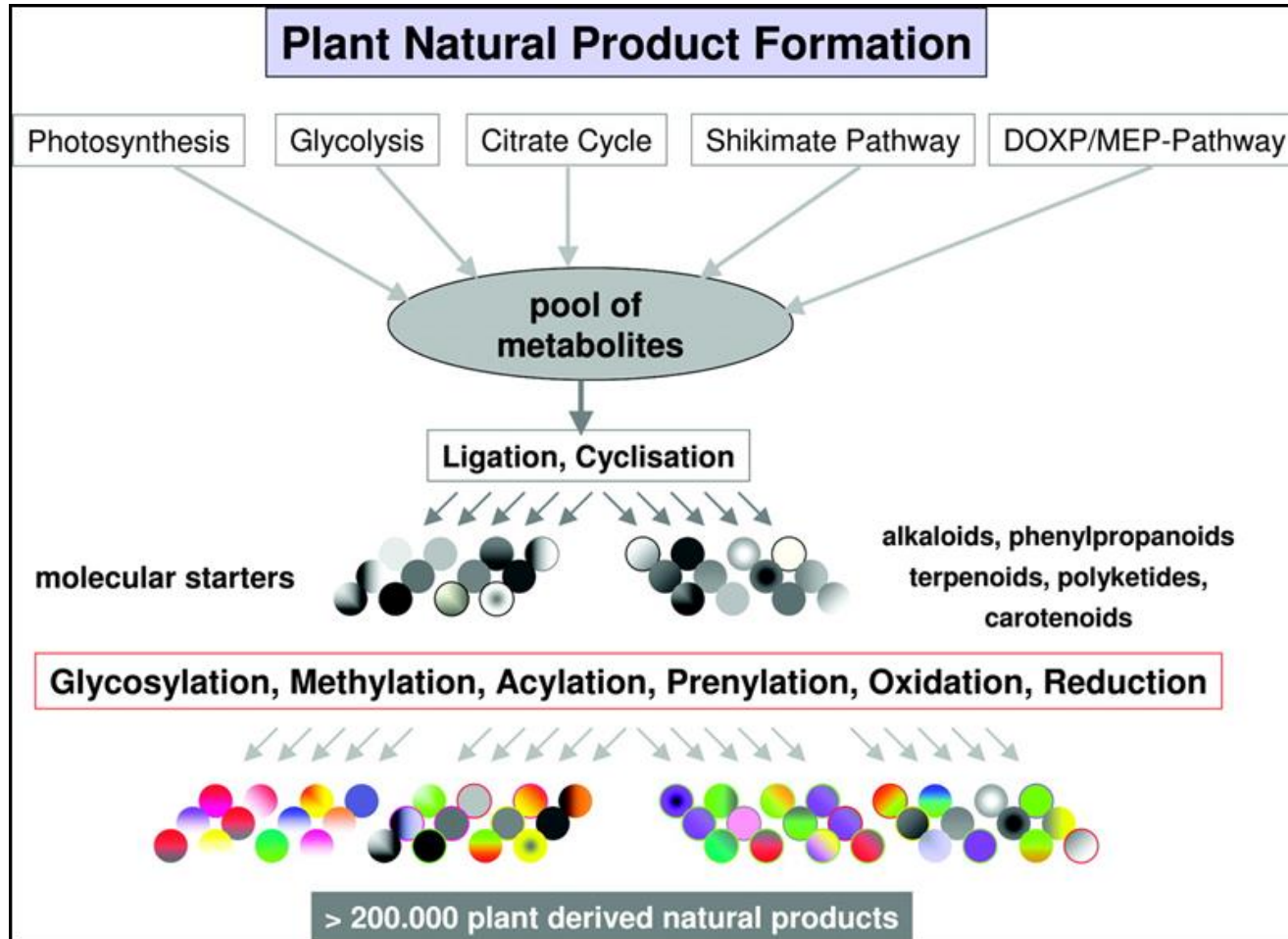
- **Primary metabolites**

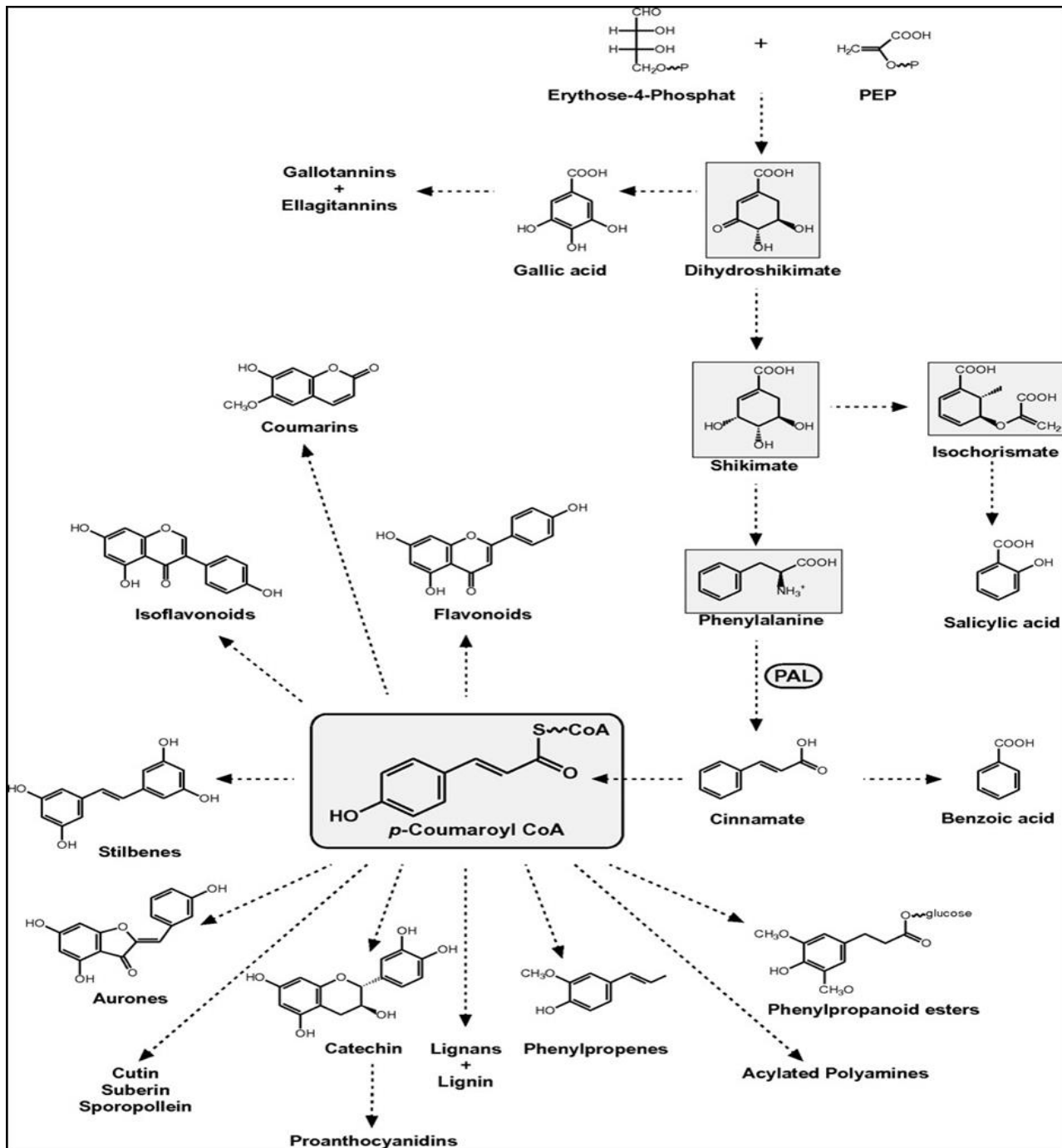
- nucleotides, amino acids, lipids and sugars

- **Secondary metabolites / Natural products**

- biosynthesis is often restricted in a certain taxonomical group
- functions are mainly in the interaction between the plant and its environment
- three major biosynthetic routes - the shikimate / phenylpropanoid, isoprenoid and polyketide pathways
- classified based on their chemical features and biosynthetic origin

Chemical diversity of primary and secondary plant metabolites





Diversity of the phenylpropanoids



Analytical platforms used in Metabolomics

- Due to highly diverse physico-chemical properties of metabolites there is currently no single analytical platform that can provide the complete analysis of the whole metabolome.
- A multi-platform approach is advantageous so as to provide maximal metabolite coverage.

Analytical techniques used in Metabolomics

Technique	Sensitivity	Throughput	Comprehensiveness
NMR	Low	Low-high	Low-high
IR	Low	High	Low
LC-NMR	Low	Low	High
LC-MS	Medium	High	High
GC-MS	High	High	High
CE-MS	High	Medium	High
LC-CE-MS	High	High	High
LC-UV	Medium-high	High	Very low

Chromatography

- GC (gas chromatography)
 - Non-polar, volatile, thermally stable compounds
 - Primary metabolites (amino acids and organic acids as volatile derivatives)
 - Volatile secondary metabolites (e.g. terpenes)
- LC (liquid chromatography, HPLC, UHPLC)
 - Polar and non-polar small organic molecules
 - Secondary metabolites

Mass spectrometry

MS instruments are composed of two main sections:

- Ionization technology
 - EI - electron ionization
 - CI - chemical ionization
 - ESI - electrospray ionization
 - APCI - atmospheric pressure chemical ionization
 - MALDI - matrix-assisted laser desorption/ionization
 - FAB - fast atom/ion bombardment
- Mass analyzer
 - Q - quadrupole
 - QQQ - triple-quadrupole
 - IT - ion-trap
 - TOF - time of flight

Reporting standards in Metabolomics

- Accurate reporting standards for metabolomics data should be followed
- Metabolomics standards initiative (MSI) to document research accurately and to enable other researchers to duplicate results
- These guidelines are not describing how to perform investigations but are rather a description as to how the experimental work should be carried out. This will also ensure that all possibilities of bias reporting are eliminated

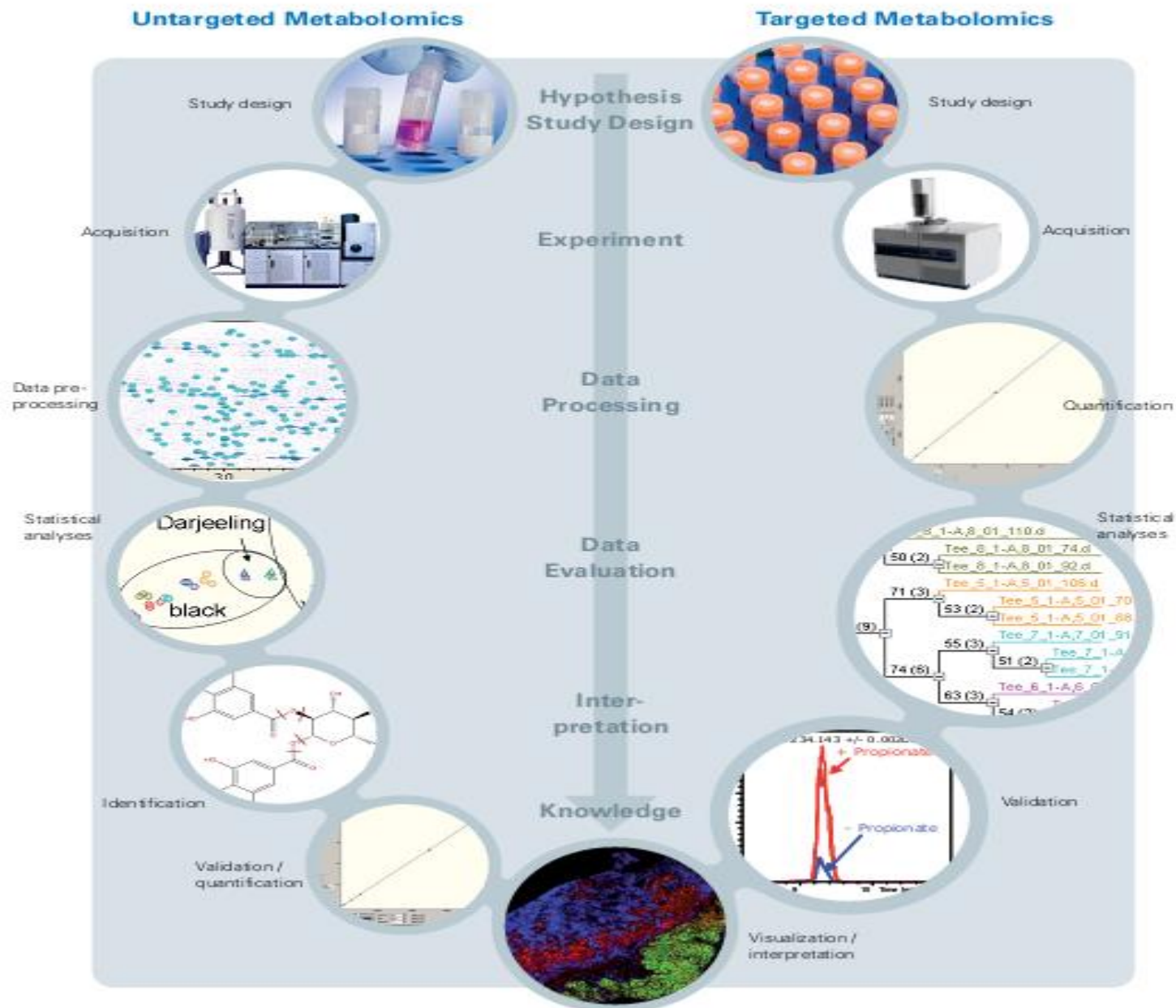
Designing Metabolomics experiments

- The design of a metabolomics experiment is very crucial so as to lead to valid and reproducible results with meaningful biological knowledge and insights.
- Take note of requirements for statistical analysis.
- The quality of samples and handling determines the quality of outcomes of the metabolite analyses.
- Consistency in sample collection and preparation is mandatory for maximum data output in metabolomic studies.

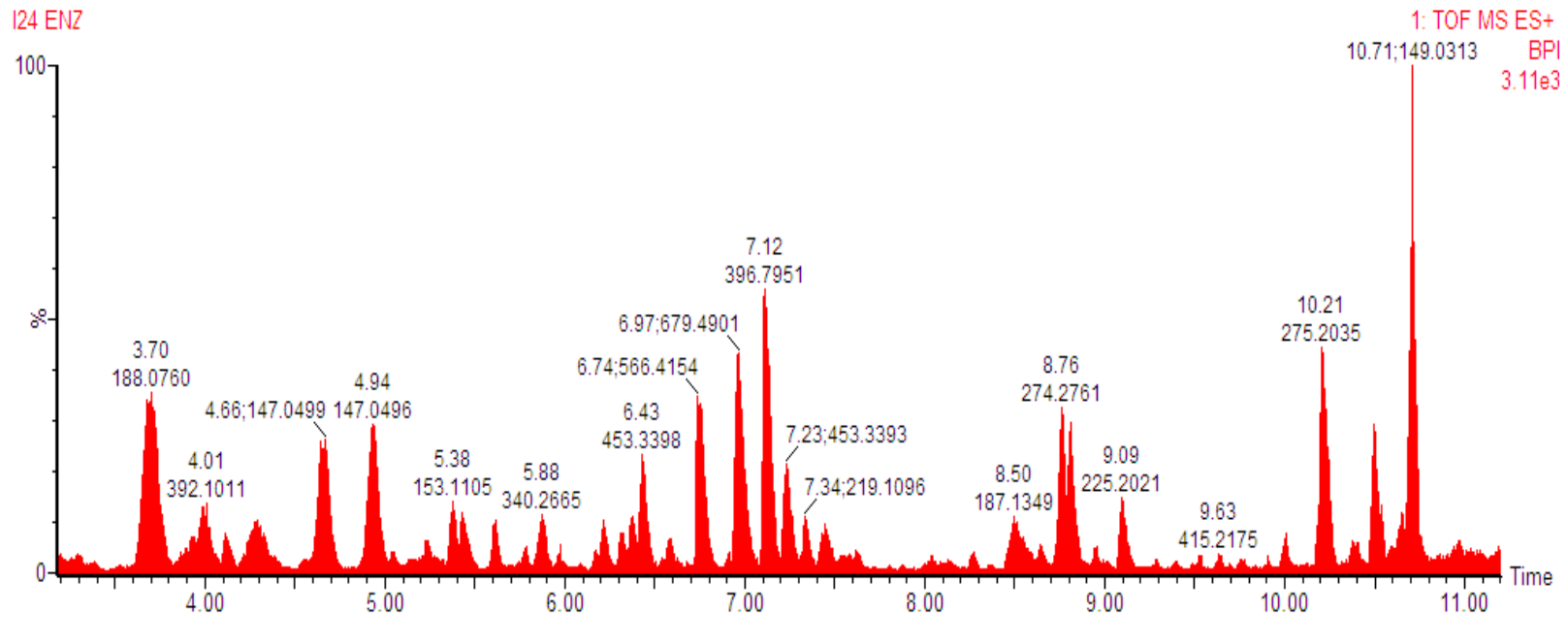
The problem of variability

- ❖ Regional differences
- ❖ Diurnal variations
- ❖ Developmental stages
- Environmental factors
- Stress factors
- Extraction methodologies
- Sample stability
- Instrumental variations

Handling the Entire Workflow



LC-MS Chromatogram

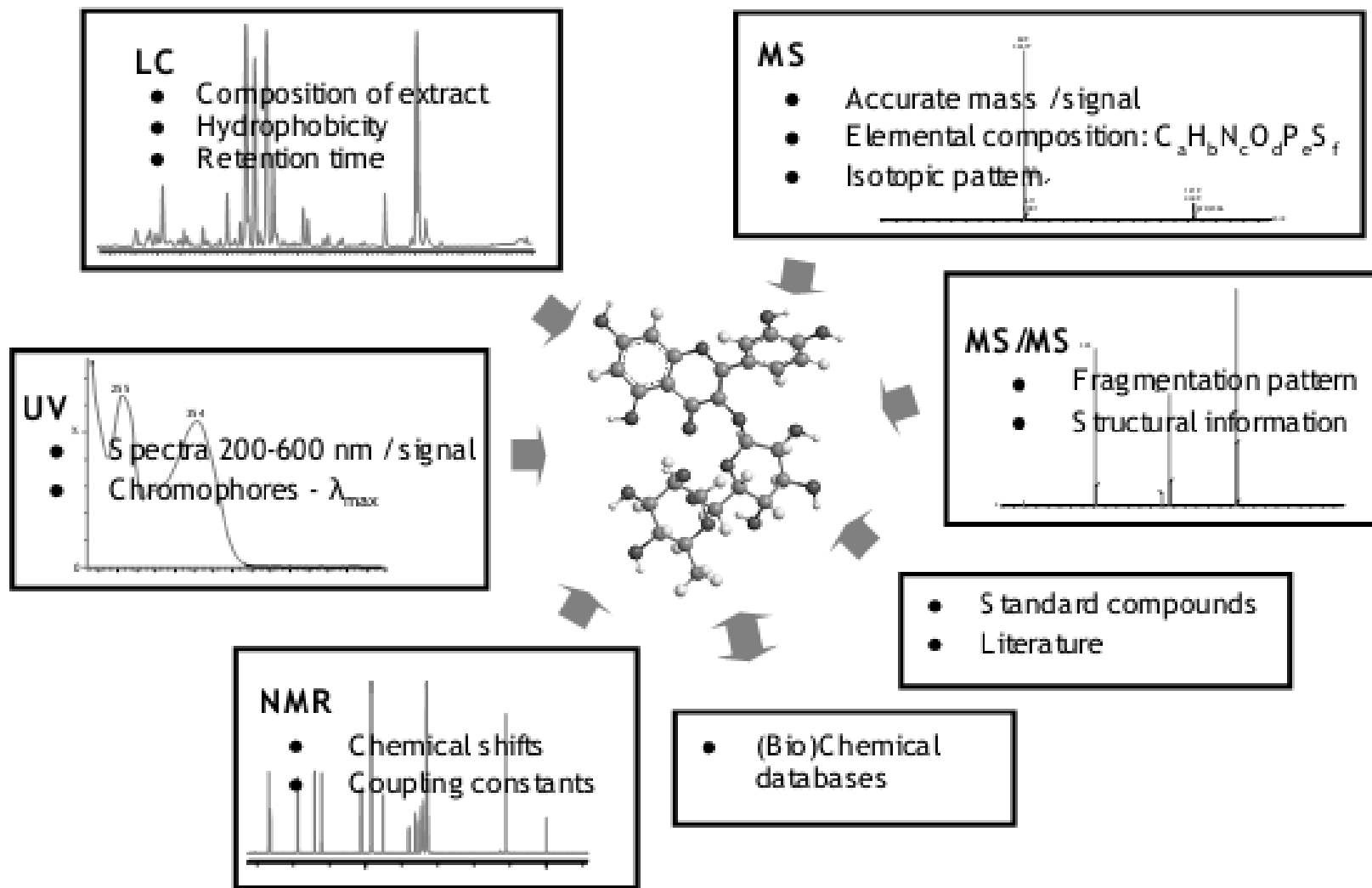


BPI = Base peak Intensity

Rt from LC

m/z from MS

Identification of signatory biomarkers



Application of metabolomics in plant sciences

- Bioactive metabolites - Medicinal plant research
- Interpretation of metabolic pathways and networks
- Biomarker discovery
 - identification of novel molecular targets
- Discovery of metabolites involved in environmental adaptations
 - Abiotic stress responses (heat, drought, cold)
 - Biotic stress and host : pathogen interactions
- Genotyping
- Gene function elucidation
- Plant breeding and crop quality assessment
- Molecular biotechnology and recombinant DNA technology
- **GMOs**
 - **Risk assessment**
 - **Substantial equivalence**