Lunch options close to the BRT

**Brenen’s Café**
Located in the lobby of the Biomedical Research Tower, Brenen’s is a full-service café offering breakfast and lunch including baked goods, salads, frozen yogurt and coffee. Visit brenensinc.com for a full menu.

**Food truck** (John H Herrick near Aronoff Labs)

**Au Bon Pain** (0.1 mile)
Located on the conference level of The James, Au Bon Pain is a full-service café offering made-to-order sandwiches, salads, baked goods, breads, hot or iced coffee and tea.

**Wendy’s** (0.2 mile)
Located on the ground floor between the 12th Avenue Garage and The Brain and Spine Hospital.

**BistrOH!** (0.3 mile)
Located on the first floor of Rhodes Hall, BistrOH!, the hospital cafeteria, features items such as flatbread pizzas, signature salads, hot and cold sandwiches and entrees.

**BistrOH! to go** (0.3 mile)
Located on the concourse of Rhodes Hall, BistrOH! to go offers quick-serve and grab-and-go items.

**Panera Bread** (0.3 mile)
Located in the SAFEAUTO Garage, Panera Bread is a full-service bakery-café with breakfast, lunch and dinner options including hot soups, fresh salads, sandwiches on freshly-baked bread, breakfast sandwiches, bagels, pastries, cookies and coffee.

**Hangover Easy**, 1646 Neil Ave (0.3 mile)
[https://www.hangovereasy.com/menu](https://www.hangovereasy.com/menu)
## Summer Workshop Schedule
### July 25-27, 2022

<table>
<thead>
<tr>
<th>Time</th>
<th>Monday, 7/25</th>
<th>Tuesday, 7/26</th>
<th>Wednesday, 7/27</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-10:00am</td>
<td>Workshop Overview, Ionization Methods (Arpad Somogyi)</td>
<td>Quantitative Proteomics (Liwen Zhang)</td>
<td>Proteomics Data Analysis (Mike Freitas, Julian Aldana Aroca)</td>
</tr>
<tr>
<td>10:15-12:00pm</td>
<td>FT-ICR (FT-MS) instrumentation and techniques (Mike Freitas)</td>
<td>Imaging (Amanda Hummon)</td>
<td>Native Mass Spectrometry (Vicki Wysocki, Sophie Harvey) Peptide Manual Sequencing (Arpad Somogyi)</td>
</tr>
<tr>
<td></td>
<td>Mass Analyzers (Arpad Somogyi)</td>
<td>New Instruments and Services (Arpad)</td>
<td></td>
</tr>
<tr>
<td>12-1pm</td>
<td>Lunch Break</td>
<td>Lunch Break</td>
<td>Lunch Break</td>
</tr>
<tr>
<td>1:00-2:00pm</td>
<td>Introduction to Proteomics (Mike Freitas)</td>
<td>HPLC MS/MS and Metabolomics Sample Preparation (Matt Bernier)</td>
<td>Peptide Manual Sequencing (continued) (Arpad Somogyi)</td>
</tr>
<tr>
<td>2:00-2:30pm</td>
<td>Sample Preparation for Proteomics and Post translation modifications (PTMs) (Liwen Zhang/Brian Fries)</td>
<td>Data Processing for Small Molecules (Matt Bernier)</td>
<td>Small Molecule Analysis (Arpad Somogyi)</td>
</tr>
<tr>
<td>2:45-4:00pm</td>
<td>Lab Visit (Group 1)</td>
<td>Open Discussion (Volunteers)</td>
<td>Lab Visit (Group 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jared Show:: High resolution ion mobility for discovery and characterization of antibody therapeutics</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ariana Shannon: Leveraging DIA acquisition to detect cellular crosstalk in cocultured tumor models</td>
<td></td>
</tr>
</tbody>
</table>

**Thanks to:**

Laura VanArsdale, and All presenters/participants
Overview

What is Mass Spectrometry?

• Mass spectrometry is a powerful tool in analytical/bioanalytical chemistry that provides
  – detailed structural information for a wide variety of compounds (MW: 1-10^6 daltons) by using
  – a small amount of sample (ug, picomol, femtomol)
  – often and easily coupled with separation techniques (GC, HPLC, IM) – *ideal for mixture analysis*
What can we provide by mass spec?

- **MW determination**
  - Nominal (mass shift due to mutations)
  - accurate (elemental composition)
    - isotope pattern
    - high resolution

- **Fragmentation**
  - fragmentation rules (peptide/protein sequencing, protein complexes)
  - libraries ("fitting") (drug metabolism, pharmacokinetics)
  - MS/MS (or MS^n) (proteomics, genomics, metabolomics)
Sample Preparation

Sample Introduction
Direct probe/infusion
GC
HPLC

Ionization Source

Vacuum System

Mass Analyzer

Detector

Computer

Electron impact (EI)
Chemical ionization (CI)
Atmospheric pressure (API)
Electrospray (ESI)
Matrix assisted laser
Desorption/ionization (MALDI)
Surface enhanced LDI (SELDI)
Fast atom bombardment (FAB)

Electrostatic (ESA)
Magnet (B)
Time-of-flight (TOF)
Quadrupole (Q)
Ion Traps (2D & 3D IT)
Ion-Cyclotron Resonance (ICR)
Orbitrap (OT)

Electron Multiplier
Photomultiplier
Faraday cap
Array Detectors
Multichannel plate

Rough,
Turbomolecular, and
Cryo pumps
Collide with target to produce fragments
Simulation of Two-Step Process
For each of the following applications, choose the most appropriate mass analyzer from the following list.

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>orbitrap (OT)</td>
<td>synthetic organic chemist wants exact mass of compound</td>
</tr>
<tr>
<td>quadrupole (Q)</td>
<td>biochemist wants protein molecular weight of relatively large protein (MW 300,000)</td>
</tr>
<tr>
<td>time-of-flight (TOF)</td>
<td>EPA (Environmental Protection Agency) wants confirmation of benzene in extracts from 3000 soil samples</td>
</tr>
<tr>
<td>FTICR</td>
<td>Petroleum chemist wants to confirm the presence of 55 unique compounds at one nominal mass/charge value in a mass spectrum</td>
</tr>
</tbody>
</table>
Mass Spectrometry Workshop
Ionization Methods

Árpád Somogyi
CCIC MSP
(Abraham Badu, Professor, Chemistry and Biochemistry)

July 25, 2022
OSU
Advances in Mass Spectrometry

**Ion Source** → **Mass Analyzer** → **Detector**

- **Electron Ionization (EI)**
- **Chemical Ionization (CI)**
  - For volatile compounds
  - (≈1% of all compound)

- **Atmospheric Pressure Chemical Ionization (APCI)**
- **Electrospray Ionization (ESI)**
  - For non-volatile liquid samples

**Ambient Ionization Methods**

**Some Sample Types**
- Human Tissue
- Banknotes
- Whole Plant
- Drug Tablet

Analyte Sampling from their Native Environment without Sample Pre-treatment

Badu-Tawiah et. al. *Annu. Rev. Phys. Chem.* 2013, 64, 481–505
Ionization Methods

Neutral species → Charged species

- Removal/addition of electron(s)
  - \( M + e^- \rightarrow (M^+.)^* + 2e^- \)
    - electron ionization

- Removal/addition of proton(s)
  - \( M + (\text{Matrix})-H \rightarrow MH^+ + (\text{Matrix})^- \)
    - Chemical ionization (CI)
    - Atmospheric pressure CI (APCI)
    - Fast atom bombardment (FAB)
    - Electrospray/nanospray ionization (nESI)
    - Matrix assisted laser desorption/ionization (MALDI)
    - Desorption electrospray ionization (DESI)
    - Direct analysis in real time (DART)
    - Native spray
Ion Sources: volatiles; non-volatile liquids; non-volatile solids

Native MS

MALDI

EESI

Fishing native proteins from biological soups
Electron Impact (EI) Ionization

Developed in 1918 by Dempster
Still widely used in
Forensic
Environmental
Drug Metabolism, etc.

EI is a gas-phase process, and requires the analyte to be volatile
Electron Ionization (EI) – hard ionization

\[ M_{(g)} + e^- \rightarrow M^{+\cdot}_{(g)} + e^- + e^- \]

70 eV

Sample inlet
Electron collector
Repeller
Source cage
filament

To analyzer

Dissociative Result
Charged and Neutral Fragments
Non-dissociative Result
Ionized Parent Molecule
Fragmentation in Electron Ionization

Consider formaldehyde: $\text{H}_2\text{C}=\text{O}$

Excess energy is deposited into the ion, which causes fragmentation.

Why 70 eV?
It gives reproducible fragmentation patterns necessary for the creation of library.

What electron is removed?

How much excess energy is unaccounted for?
The ion source. Ions are generated by bombarding gaseous molecules with a beam of high energy electrons.

\[ M + e^- \rightarrow (M^+)^* + 2e^- \]

Unimolecular fragmentation

\[
\begin{align*}
(M^+)^* & \rightarrow F_{11} \rightarrow F_{12} \quad \ldots \\
& \rightarrow F_{21} \rightarrow F_{22} \quad \ldots \\
& \rightarrow F_{31} \rightarrow F_{32} \quad \ldots
\end{align*}
\]

Mass spectrum: the result of consecutive and competitive processes
The Mass Spectrum

The mass spectrum of acetone, \( \text{CH}_3\text{COCH}_3 \), contains many fragment ions as well as the molecular ion at m/z 58.

\[
\begin{align*}
\text{CH}_3\text{CCH}_3 + e^- & \rightarrow (\text{CH}_3\text{CCH}_3)^* + 2e^- \\
E_a^{(s)} & \rightarrow \text{CH}_3\text{C} = \text{O}^+ + \cdot \text{CH}_3 \\
E_a^{(z)} & \rightarrow \text{CH}_3\text{C} = \cdot \text{O}^+ + \text{CH}_3
\end{align*}
\]

- molecular ion (M\(^{+}\))
- fragment ions (F\(^{+}\))
- metastable ions (discussed later)
- base peak (m/z 43)
A Useful Theoretical Insight

M + e^- → M^+ + e^- + e^-

Excess gained ≈ 4 eV
Statistically Distributed

OH
Phenol
MW 94 Da

m/z 77
Direct bond cleavage

H_2O
m/z 76
Rearrangement

Energy
Electron Impact
Dissociation Coordinate

+ 2 electrons
+ 2 electrons
Internal Energy Distribution $P(E)$ and $k(E)$ Curves

Direct bond cleavage
Rearrangement

RRK (simplified)

$$k(E) = (1 - E_0/E)^{s-1}$$

Critical Energy
Figure 3.3. Mass spectrum of hexyl ether.
TABLE 1.2
Advantages and Disadvantages of Electron Ionization

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subpicomole to picomole sensitivity.</td>
<td>Limited mass range due to thermal desorption (volatility) requirement.</td>
</tr>
<tr>
<td>Availability of vast computer databases, containing over 100,000 compounds.</td>
<td>Possible decomposition by thermal desorption prior to vaporization.</td>
</tr>
<tr>
<td>Use of fragmentation pattern as a fingerprint with databases to identify</td>
<td>Too much fragmentation, often resulting in no observable molecular ion.</td>
</tr>
<tr>
<td>unknowns.</td>
<td></td>
</tr>
<tr>
<td>Structural information obtained from fragmentation pattern.</td>
<td></td>
</tr>
</tbody>
</table>

\[
M_{(g)} + e^- \rightarrow M_{(g)}^{+} + e^- + e^- \\
\text{→ hard ionization}
\]

\[\text{heat} \quad 70 \text{ eV} \]

\[M_{(s)}\]
Chemical Ionization – Soft Ionization

- Ion-molecule reaction(s) between a reagent gas and a gas-phase analyte at a relatively high pressure

- Most common reagent gases
  - methane, isobutane, ammonia

- Mechanisms
  - $\text{CH}_4 + \text{e}^- (70 \text{ eV}) \rightarrow \text{CH}_4^+, \text{CH}_3^+, \text{CH}_2^+, \ldots$
  - $\text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3$
  - $\text{CH}_3^+ + \text{CH}_4 \rightarrow \text{C}_2\text{H}_5^+ + \text{H}_2$
  - $\text{CH}_5^+ + \text{M} \rightarrow [\text{M+H}]^+ + \text{CH}_4$
  - $\text{C}_2\text{H}_5^+ + \text{M} \rightarrow [\text{M-H}]^+ + \text{C}_2\text{H}_6$
  - $\text{C}_2\text{H}_5^+ + \text{M} \rightarrow [\text{M+ C}_2\text{H}_5]^+$
Figure 11
Electron ionization of ephedrine at 70 eV. No molecular ion is seen (m/z 165).

Methane chemical ionization mass spectrum of ephedrine. The protonated molecule is seen at m/z 166.
Types of Reactions Used for CI

- **Proton transfer**: \( M + BH^+ \rightarrow MH^+ + B \)
- **Charge exchange**: \( M + X^+ \rightarrow M^+ + X \)
- **Electrophilic addition**: \( M + X^+ \rightarrow MX^+ \)
- **Anion abstraction**: \( AB + X^+ \rightarrow B^+ + AX \)
Protonation is one type of ionization

\[ \text{M} + \text{AH}^+ \rightarrow \text{MH}^+ + \text{A} \]

\[ \text{CH}_3\text{CH}_2\text{NH}_2 + \text{NH}_4^+ \rightarrow \text{CH}_3\text{CH}_2\text{NH}_3^+ + \text{NH}_3 \]

Reaction proceeds only if it is Thermoneutral or exothermic (i.e., \( \Delta H_r \leq 0 \))

\[ \Delta H_r = \Delta PA = \text{PA (reagent)} - \text{PA (analyte)} \]

For negative \( \Delta PA \), \( \text{PA (analyte)} > \text{PA (reagent)} \)

\[ \text{i.e., energy will be released!} \]

--

Proton affinity (PA):

\[ \text{M} + \text{H}^+ \rightarrow \text{MH}^+ \]
Proton affinity (PA):

PA of common CI reagents (kcal/mol)
methane (131) < water (173) < methanol (185) < \( \text{CH}_2=\text{C(CH}_3\text{)}_2 \) (197) < ammonia (205)

→ energy released is controllable via proper selection of reagents

→ energy released (≈ΔPA) is absorbed by analyte

→ extend of fragmentation is also controllable
CIMS: Two different reagent gases.

Figure 10. Chemical ionization spectra of diisooctyl phthalate (mass 390) using (a) methane and (b) isobutane reagent gases.\textsuperscript{14}
Major Limitation of EI and CI

Requires volatility, good for thermally stable compound (<1% of all compounds)

Solution: develop desorption and spray ionization

\[
M_{(g)} + e^- \rightarrow M^+_{(g)} + e^- + e^-
\]

(reagent)

heat

70 eV

M_{(s)}

(not applicable to large molecules)
Can we teach elephants to fly?

How important will this be?

John Fenn
How important?
Nobel Price in Chemistry, 2002

John B. Fenn

Koichi Tanaka
ESI and MALDI for Biological Systems

Spray Ionization

↑

Soft

works well for large molecules

↓

Desorption Ionization

ESI

MALDI
**Matrix-Assisted Laser Desorption Ionization**

MATRIX - A small acidic organic molecule (matrix) is mixed with a low concentration of analyte in a common solvent and allowed to co-crystallize on a sample plate to form a “solid solution” by which the analyte molecules are isolated from each other.

ASSISTED – matrix “assists” the desorption and ionization of the analyte(s)

LASER – Typically a Nitrogen laser (351 nm) or Yag/Nd laser (334 nm)

DESORPTION – Energy from the laser desorbs the matrix into the gas-phase and “carries” the analyte with it.

IONIZATION - Detect \[\text{M+H}^+\] by transferring a proton from the matrix to the analyte

- Choose a matrix based on the molecular weight, solubility and chemical structure of the analyte.
- Excellent for intact molecular weight determination for both polar and non-polar molecules with mass > 500 amu.
Hypothesis/speculations about Ionization

• Disintegration of solid:
  – Statistical charging of clusters in a net neutral plume
  – Total charge due to
    • Charge separation of “salts”
    • Photoionization of matrix

• Chemical Ionization
  – Charged clusters generated in selvedge evaporate and undergo
    • Ion-neutral reactions,
    • Ion-ion reactions
    • Ion-electron reactions
Table 2. PA values (kcal/mol) of MALDI matrices given in the present study and comparison of the same with literature values

<table>
<thead>
<tr>
<th>Matrix compound</th>
<th>Reference bases used</th>
<th>2 eV</th>
<th>5 eV</th>
<th>7 eV</th>
<th>10 eV</th>
<th>Proton affinity(^a)</th>
<th>Average T(_{off}) in K</th>
<th>Jorgensen et al. [31]</th>
<th>Burton et al. [32]</th>
<th>Steenvoorden et al. [34]</th>
<th>Nelson et al. [33]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4HCCA</td>
<td>1-4</td>
<td>201.5</td>
<td>201.0</td>
<td>200.9</td>
<td>200.6</td>
<td>201.0 ± 0.27 (±0.64)</td>
<td>817</td>
<td>201.0</td>
<td>183.0</td>
<td>223.0</td>
<td>203.0</td>
</tr>
<tr>
<td>GA</td>
<td>2-6</td>
<td>204.7</td>
<td>204.3</td>
<td>204.3</td>
<td>204.3</td>
<td>204.4 ± 0.17 (±0.36)</td>
<td>528</td>
<td>-</td>
<td>204.0</td>
<td>204.0</td>
<td>202.9</td>
</tr>
<tr>
<td>MSA</td>
<td>3-5,8</td>
<td>205.3</td>
<td>205.2</td>
<td>205.2</td>
<td>205.1</td>
<td>205.2 ± 0.52 (±1.22)</td>
<td>399</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SA</td>
<td>7,9-11</td>
<td>209.3</td>
<td>209.2</td>
<td>209.2</td>
<td>209.2</td>
<td>209.2 ± 0.38 (±0.89)</td>
<td>427</td>
<td>212.0</td>
<td>204.0</td>
<td>214.0</td>
<td>210.0</td>
</tr>
<tr>
<td>DT</td>
<td>10,12-14</td>
<td>211.5</td>
<td>211.4</td>
<td>211.6</td>
<td>211.6</td>
<td>211.5 ± 0.77 (±1.81)</td>
<td>507</td>
<td>-</td>
<td>209.0</td>
<td>-</td>
<td>-</td>
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<tr>
<td>AMT</td>
<td>10,12-14</td>
<td>213.1</td>
<td>213.0</td>
<td>212.9</td>
<td>213.0</td>
<td>213.0 ± 0.31 (±0.73)</td>
<td>515</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>THAP</td>
<td>10,12-14</td>
<td>213.3</td>
<td>213.4</td>
<td>213.2</td>
<td>213.0</td>
<td>213.3 ± 0.65 (±1.53)</td>
<td>576</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>210.8</td>
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<tr>
<td>IAA</td>
<td>10,12-14</td>
<td>214.0</td>
<td>213.6</td>
<td>213.3</td>
<td>212.9</td>
<td>213.5 ± 0.26 (±0.61)</td>
<td>772</td>
<td>-</td>
<td>215.0</td>
<td>-</td>
<td>-</td>
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<tr>
<td>HPA</td>
<td>9-11,14</td>
<td>214.9</td>
<td>214.7</td>
<td>214.5</td>
<td>214.2</td>
<td>214.6 ± 0.33 (±0.78)</td>
<td>714</td>
<td>214.0</td>
<td>-</td>
<td>-</td>
<td>214.5</td>
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<tr>
<td>MBT</td>
<td>10-12-14</td>
<td>214.9</td>
<td>214.9</td>
<td>214.9</td>
<td>214.9</td>
<td>214.9 ± 0.23 (±0.54)</td>
<td>655</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>AAMT</td>
<td>10-12,14</td>
<td>215.5</td>
<td>215.7</td>
<td>215.8</td>
<td>215.8</td>
<td>215.7 ± 0.14 (±0.33)</td>
<td>619</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>EMT</td>
<td>15-19</td>
<td>218.1</td>
<td>218.0</td>
<td>217.9</td>
<td>217.9</td>
<td>218.0 ± 0.17 (±0.36)</td>
<td>432</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MP</td>
<td>15-19</td>
<td>219.6</td>
<td>219.5</td>
<td>219.5</td>
<td>219.5</td>
<td>219.5 ± 0.19 (±0.41)</td>
<td>402</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HABA</td>
<td>18-21</td>
<td>227.3</td>
<td>226.9</td>
<td>226.7</td>
<td>226.6</td>
<td>226.9 ± 0.26 (±0.61)</td>
<td>556</td>
<td>225.0</td>
<td>183.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NH</td>
<td>22-25</td>
<td>233.1</td>
<td>233.1</td>
<td>233.0</td>
<td>232.8</td>
<td>233.0 ± 0.44 (±1.03)</td>
<td>395</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)Average of standard deviation at different collision energies. Values in parentheses are uncertainties at 90% confidence limits [49].
Sample and matrix are spotted on MALDI plate
MALDI instrument

MALDI is a vacuum-based ionization method
Often coupled to Time-of-Flight Instrument
### MALDI Matrix Materials

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular Structure</th>
<th>Molecular Formula</th>
<th>Monoisotopic Mass [M+H]^+</th>
<th>Average Mass [M+H]^+</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-hydroxypicolinic acid (3-hydroxy-2-pyridinecarboxylic acid)</td>
<td><img src="image1" alt="Molecular Structure" /></td>
<td>C₇H₅NO₃</td>
<td>140.0347</td>
<td>140.119</td>
</tr>
<tr>
<td>Nicotinic acid-N-oxide</td>
<td><img src="image2" alt="Molecular Structure" /></td>
<td>C₇H₅NO₃</td>
<td>140.0347</td>
<td>140.119</td>
</tr>
<tr>
<td>2′-6′-dihydroxyacetophenone</td>
<td><img src="image3" alt="Molecular Structure" /></td>
<td>C₈H₅O₃</td>
<td>153.0552</td>
<td>153.158</td>
</tr>
<tr>
<td>Gentisic acid (2,5-dihydroxybenzoic acid)</td>
<td><img src="image4" alt="Molecular Structure" /></td>
<td>C₇H₆O₄</td>
<td>155.0344</td>
<td>155.130</td>
</tr>
<tr>
<td>α-cyano-4-hydroxycinnamic acid</td>
<td><img src="image5" alt="Molecular Structure" /></td>
<td>C₁₀H₇NO₃</td>
<td>190.0502</td>
<td>190.178</td>
</tr>
<tr>
<td>Ferulic acid (4-hydroxy-3-methoxybenzoic acid)</td>
<td><img src="image6" alt="Molecular Structure" /></td>
<td>C₁₀H₈O₄</td>
<td>195.0657</td>
<td>195.195</td>
</tr>
<tr>
<td>Sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid)</td>
<td><img src="image7" alt="Molecular Structure" /></td>
<td>C₁₁H₉O₃</td>
<td>225.0763</td>
<td>225.222</td>
</tr>
</tbody>
</table>
## MALDI Matrices

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Abbrev</th>
<th>Sample Type</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,5-dihydroxybenzoic acid</td>
<td>DHB</td>
<td>Peptides &lt; 5,000 polymers, dedrimers</td>
<td>50% ACN in 0.1% TFA, THF, 2:1 chloroform:MeOH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Good universal matrix “cold matrix”</td>
<td></td>
</tr>
<tr>
<td>3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic acid)</td>
<td>SA</td>
<td>Peptides and Proteins &gt; 10,000 “hot matrix”</td>
<td>50-70% ACN in 0.1% TFA</td>
</tr>
<tr>
<td>α-cyano-4-hydroxycinnamic acid</td>
<td>HCCA</td>
<td>Excellent for peptides, digestion products and proteins</td>
<td>50% ACN in 0.1% TFA</td>
</tr>
<tr>
<td>Dithranol</td>
<td></td>
<td>Non-polar polymers</td>
<td>THF, Methylene chloride</td>
</tr>
<tr>
<td>Indoleacrylic acid</td>
<td>IAA</td>
<td>Non-polar polymers</td>
<td>THF, methylene chloride</td>
</tr>
<tr>
<td>3-hydroxypicolinic acid</td>
<td>HPA</td>
<td>DNA and negative ion samples</td>
<td>See me for more specific procedure</td>
</tr>
<tr>
<td>Trihydroxyacetophenone</td>
<td>THAP</td>
<td>DNA and negative ion samples</td>
<td>See me for more specific procedure</td>
</tr>
<tr>
<td>Nor-harmane</td>
<td></td>
<td>Universal</td>
<td>50% ACN, THF, chloroform</td>
</tr>
</tbody>
</table>
MALDI Matrices: common features?

*trans*-cinnamic acid

2,5-dihydroxybenzoic acid

Alpha-cyano-4-hydroxy cinnamic acid
MALDI Matrices: aromatic to absorb UV

trans-cinnamic acid

2,5-dihydroxybenzoic acid

Alpha-cyano-4-hydroxy cinnamic acid
Protein Analysis by MALDI-TOF MS

Why are singly charged ions predominantly formed in MALDI? “Lucky survivor model”
High m/z can be observed with MALDI - TOF

Resolution modest, no sequence info

Alkaline Phosphatase

\[ [M+H]^+ \]
\[ 47155.22 \]

\[ [M+2H]^2+ \]
\[ 23445.54 \]

\[ [2M+H]^+ \]
\[ 94472.97 \]
MALDI Spectrum from Nicotinic Acid Matrix of Monoclonal Antibody (IgG)

Monoclonal antibody (IgG)

Intensity, relative units

$M^{++}$

$M^{+++}$

$2M^{+++}$

$3M^{++}$

$2M^+$

$M^+$: 149190
Washing sample after crystallization with the matrix

Human hemoglobin alpha & beta chains
MALDI of peptide mixture
(Also works for these "lower" MW)
MALDI Matrix-Assisted Laser Desorption Ionization

- Protein analyzed from crystalline matrix; separation performed off-line
- Produces mainly 1+ or 2+ ions
- More tolerant to salt
- Mainly a MS technique until recently Pulsed laser complicates MS/MS
MALDI works well for polymers

http://www.psrc.usm.edu/mauritz/maldi.html
Synthetic Polymer Analysis by MS (MALDI-TOF)

- Intens. [a.u.]
- m/z

- Peaks identified:
  - 1684.205
  - 1728.231
  - 1772.260
  - 1816.292
  - 1660.310
Repeating unit masses of polymers

http://www.polymerprocessing.com/polymers/alpha.html

- [ CH₂ - CH ] -

Polystyrene (PS)
C₈H₈ (104.062600)

Poly(ethylene terephthalate) (PET)
C₁₀H₈O₄ (192.042259)

- [ O - (CH₂)₂ - O - C = O ] -

Poly(methyl methacrylate) (PET)
C₅H₈O₂ (100.052430)

- [ CH₂ - CH ] -

Poly(vinyl alcohol) (PVA)
C₂H₄O (44.026215)

- CH₂-CH₂-O-

Poly(ethylene glycol) (PEG)
Molecular weight distribution defines polydispersity of polymers


\[ M_n = \frac{\sum (N_i M_i)}{\sum N_i} \]

\[ M_w = \frac{\sum (N_i M_i^2)}{\sum N_i M_i} \]

Polydispersity (PD) = \( M_w / M_n \)

Polystyrene with Ag⁺

PD ≈ 1.01

Truncated Vectra Polymer

(Somogyi et. al., Macromolecules, 2005, 38, 4067-4071)

PD > 1.05
Problems with polymer analysis

- Sample preparation
  - Several polymers are not well soluble in conventional solvents
  - Solventless technique
  - Cationizing with Na\(^+\), K\(^+\), and Ag\(^+\) by spiking
  - Synthesis of tailor (home) made matrices

- Degradation during ionization (especially with MALDI)
  - Photodissociation in MALDI (MALDI/ESI comparison desirable)
Some Examples for Tailor-Made Matrices

ESI and MALDI for Biological Systems

Spray Ionization

Soft works for large molecules

Desorption Ionization
## Spray Ionization Techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrospray (ESI)</td>
<td>Atm</td>
</tr>
<tr>
<td>Ion spray</td>
<td>Atm</td>
</tr>
<tr>
<td>Nano spray (nESI)</td>
<td>Atm</td>
</tr>
<tr>
<td>Sonic spray (SSI)</td>
<td>Atm</td>
</tr>
<tr>
<td>Electrosonic spray (ESSI)</td>
<td>Atm</td>
</tr>
<tr>
<td>Thermospray</td>
<td>1-10 Torr</td>
</tr>
<tr>
<td>Electrohydrodynamic Ionization (EHI)</td>
<td>&lt;10^{-3} Torr</td>
</tr>
</tbody>
</table>
Electrospray Ionization

- Formation of spray
- Desolvation and breakup of droplets
- Emergence of ions from charged droplets
Generation of Stable Spray

Solvent and Voltage Effects

Solutions with electrolyte concentrations > 10^{-7} M

low voltage ~1-2 kV onset voltage

<table>
<thead>
<tr>
<th>solvent</th>
<th>CH₃OH</th>
<th>CH₃CN</th>
<th>(CH₃)₂SO</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ (N/m)</td>
<td>0.0226</td>
<td>0.030</td>
<td>0.043</td>
<td>0.073</td>
</tr>
<tr>
<td>E_{on} (Volt)</td>
<td>2200</td>
<td>2500</td>
<td>3000</td>
<td>4000</td>
</tr>
</tbody>
</table>

*calculated for a spray tip of 0.1 mm radius at D = 4 cm
Electrospray Ionization (ESI)

Metal Capillary
3-5 kV

Fused silica

Evaporation

Columbic Explosion

Critical Radius
Charge Repulsion > Surface Tension

Fused silica

Taylor cone

Jet

Needle tip

Plume
Figure 1.8 Ion formation from electrospray ionization source.
ESI-MS Example: Hen Egg Lysozyme

1:1 (v/v) acetonitrile : 0.1% aqueous formic acid
Multiple Charging in Electrospray Ionization

Figure 1.9 The same protein with a molecular weight of 10,000 contains 5, 4, 3, 2, and 1 charges. The mass spectrometer detects the protein ions at $m/z = 2001$, 2501, 3334, 5001, and 10,001, respectively.
Interpretation of ESI-Mass Spectra

\[ \frac{m}{z} = \frac{\text{MW}}{n} + nH^+ \]
Interpretation of ESI-Mass Spectra

\[ \frac{m}{z} = \frac{MW + nH^+}{n} \]

\[ \frac{m}{z} = \frac{MW + (n + 1)H^+}{(n + 1)} \]
ESI-MS Example: Hen Egg Lysozyme

1:1 (v/v) acetonitrile : 0.1% aqueous formic acid
Interpretation of ESI-Mass Spectra (cont’d)

1431.6 = \frac{MW + nH^+}{n} \quad 1301.4 = \frac{MW + (n+1)H^+}{(n+1)}

n (1431.6) - nH^+ = (n + 1) 1301.4 - (n + 1) H^+

n (1431.6 - 1301.4) = 1301.4 - H^+

\therefore n = \frac{(1301.4 - H^+)}{(1431.6 - 1301.4)} = \frac{1300.4}{130.2} = 10

Use 1431.6 = \frac{MW + nH^+}{n} to solve for MW:

1431.6 \times 10 = MW + (10 \times 1.008)

MW = 14,316 - 10.08

\therefore MW = 14,305.9 \text{ Da} \quad \text{(theoretical: 14,305.1438 Da)}

\text{(difference of 0.005%)}
ESI-MS of Myoglobin

m/z deconvolutes to 16952
ESI  Electrospray Ionization

- Protein analyzed from solution; couple to on-line separation, e.g., HPLC
- Produces multiply-charged ions
- Preserves non-covalent interactions
- Less tolerant to salts
ESI vs MALDI

Choose MALDI when:
- Peptides or protein with a MW < 5000 Da (MALDI can detect MW > 100KDa, but not very accurately)
- Complex mixtures (more than 5 compounds)
- Very little material with higher salt/buffer concentration

Choose ESI when:
- MW > 5000 Da (proteins)
- Want better mass assignment
- Want good MS/MS data
Dual ionization mode on the Solarix XR

Apollo™ II with a dual ESI/MALDI configuration
Bruker 15 T FT-ICR
Sources still being developed
DESI- desorption ESI
(sample not in solution)
DART

Solvent

HV power supply

N₂

Nebulizer capillary

Spray capillary

Gas jet

Spray

Surface

Sample

Atmospheric inlet of mass spectrometer

Ion transfer line

Desorbed ions

Freely moving sample stage in air
DART
Direct Analysis in Real Time

Penning Ionization \(M^* + S \rightarrow S^+ + M + \text{electron} \) (but also allows \(MH^+, \text{ M-H-}, \text{ etc}\))