Applications of Desorption Electrospray Ionization (DESI)

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What are the identities of $I_1$ and $I_2$?

Identifying intermediates is critical for understanding chemical reactivity.
A + B → I
1 → I
2 → P

Mass spectrometry (MS) overcomes many of these limitations

METHODS
• Optical Spectroscopy
• NMR
• Crystallography
• …

CHALLENGES
• Short timescales
• Low concentrations
• Complex systems
• Side reactions

DISADVANTAGES OF THESE METHODS
• Relatively long timescales
• Poor sensitivity
• Require relatively pure samples
GOAL
Develop a simpler and cleaner approach for studying reaction mechanisms with millisecond time resolution

DISADVANTAGES OF THESE METHODS
• Not easily amenable to high-throughput
• Carry-over effects
Desorption Electrospray Ionization (DESI)

- Ionization in open environment → Ambient MS
- No sample preparation
- High throughput
- Simple

Using DESI to Access the Millisecond Timescale

\[ A + B \rightarrow I_1 \rightarrow I_2 \rightarrow P \]

Reaction occurs in solution!

Quenched

Phase Transfer

Ru (II)-Promoted Hydrogen Transfer

Ru Complex + \( \beta \)-Amino Alcohol → Ru-Cl

\[
\text{Ru Complex: } \quad \begin{array}{c}
\text{Ru}_3 \quad \text{Cl}_3 \\
\text{Ru}_3 \quad \text{Cl}_3 \\
\text{Ru}_3 \quad \text{Cl}_3
\end{array}
\]

\[
\text{\( \beta \)-Amino Alcohol: } \quad \begin{array}{c}
\text{HO-} \\
\text{H}_2\text{N}
\end{array}
\]

\[
\text{CH}_3\text{OH} \quad \text{Ru-Cl: } \quad \begin{array}{c}
\text{Cl} \\
\text{Ru} \\
\text{Cl}
\end{array}
\]

\[\begin{align*}
+ \text{Na}^+ &= m/z 634.8921 \\ 
(1.4 \text{ ppm}) \\
+ \text{H}^+ &= m/z 150.0913 \\
(0.4 \text{ ppm}) \\
+ \text{H}^+ &= m/z 420.0670 \\
(1.3 \text{ ppm})
\end{align*}\]

Previous bulk ESI-MS studies identified these species. Structures supported by x-ray crystallography results using diamines.

Ru-amide + Ru-H

Reaction completes in a few seconds

CTH by DESI

β-Amino Alcohol

Ru Complex

Ru-amide + Ru-H

- First intermediate of the reaction!!

Confirming Peak Assignments

Orbitrap Mass Spectra

\[ \frac{m}{\Delta m} = 80,000 \]

Mass accuracy = 0.3 ppm

Isotope Ratio

%Error < 5%


Ru-amide + Ru-H

\[ \text{Relative Intensity} \]
\[ \text{m/z} \]

\[ \Delta m/z = 12 \text{ Da} \]

\[ \text{Another intermediate!!} \]

Relative $-\text{NH}_2$ and $-\text{OH}$ positions are necessary for reactivity

No CTH intermediates observed when $\text{NH}_2$ is farther away from $-\text{OH}$

• Detected species are relevant to the CTH reaction
• DESI can intercept intermediates on short timescales!!

These species are present regardless of the ligand.

Intermediates Formed from Reaction with the Solvent

Provides additional evidence that DESI can intercept rapid reaction steps

Perry et al. manuscript in preparation
Confirmation of the intermediates formed with methanol

Perry et al. manuscript in preparation
Confirmation of the intermediates formed with H$_2$O

Perry et al. manuscript in preparation
Summary

• Indicates an associative mechanism
• Observe step-wise breakdown of the dimer
• Intermediates with CH$_3$OH and H$_2$O observed
• Detected species agree with proposed catalytic mechanisms

Perry et al. manuscript in preparation
What can we learn about transfer hydrogenation catalysis?
What are the identities of $m/z$ 444 and $m/z$ 460?

Perry et al. manuscript in preparation
Ru-Methyl Formate Intermediates

- Oxidative dehydrogenation of methanol to generate methyl formate
- Adventitious O₂ oxidizes Ru (II) to Ru (IV)

Perry et al. manuscript in preparation
Perry et al. manuscript in preparation
Oxidation of Ru (II) to Ru (IV) by Adventitious O$_2$

Perry et al. manuscript in preparation
MS/MS

Normalized Collision Energy = 40%

a) MS$^2$ of m/z 444

b) MS$^2$ of m/z 460

Perry et al. manuscript in preparation
Putting it all together...

Perry et al. manuscript in preparation
What we discovered...

- DESI can intercept intermediates on short timescales (millisecond regime)

- Detected new Ru (II) reaction intermediates in transfer hydrogenations catalyzed by (β-amino alcohol)(arene)Ru complexes

- Detected intermediates formed in the reaction with CH$_3$OH and H$_2$O

- Detected a Ru-methyl formate species that suggests formation of methyl formate in CTH
Moving forward…

Combining the timescales of DESI and ESI provides a more complete picture of reaction pathways.
Many of the proposed intermediates of these catalytic systems short-lived and have not been directly observed
C-H Amination

<table>
<thead>
<tr>
<th>Compound</th>
<th>[M]⁺ m/z (ppm)</th>
<th>[M+Na]⁺ m/z (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>758.0808 (2.6)</td>
<td>781.0724 (0.1)</td>
</tr>
<tr>
<td>2</td>
<td>249.8863 (2.6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>344.9591 (1.1)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>451.8144 (1.0)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1186.9057 (2.4)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>383.9959 (1.6)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1006.9618 (0.7)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1615.7342 (0.3)</td>
<td></td>
</tr>
</tbody>
</table>

Nitrenoid reacts through an H-atom abstraction pathway

Cahill, Perry, Roizen, Davis, Du Bois, and Zare. manuscript in preparation.
High-Throughput Screening of Catalytic Mechanisms

Air-sensitive reactions

Catalyst Screening

Ligand Screening

Ru-amide + Ru-H

Blank

Ru-Cl

m/z

300 400 500 600

Perry et al. manuscript in preparation.
DESI-MS and Electrocatalysis

- This electrocatalytic reaction results in methanol oxidation
- Transfer hydrogenation catalysts can store and release energy for the electron economy

GOAL
Probe catalytic reactions on surfaces in real-time

K. R. Brownell et al. manuscript in preparation.
Another application of DESI...
Deciphering the relationship between lipids and the c-MYC gene network...
The Many Faces of c-MYC

It is critical to understand the mode of action of MYC

Studying c-MYC Expression in the Body

Conditional Transgenic Mouse Models

Tetracycline System

Doxycycline (dox)

Outcomes of c-MYC Inactivation

What are the factors and mechanisms that lead to these different outcomes?

- b) Complete reversion
- a) Partial regression
- c) Differentiation
- e) Apoptosis
- d) Arrest
- f) Loss of signals to normal cells

MYC ON

MYC OFF

c) Differentiation
d) Arrest
e) Apoptosis
f) Loss of signals to normal cells

Normal

Tumor

felsher, D. W. Cancer Res. 2008, 68, 3081
Outcomes Depend on Tissue Type

How does the tissue location affect the mechanism of c-MYC-induced cancer progression and regression?

Felsher, Cancer Res. 2008, 68, 3081
What is the relationship between lipids and the c-MYC oncogene network?
A simple approach to the problem…maybe…

**Spatial Distribution**
Distinguish lipids that are specific to tumors and normal tissue

- Wild Type
- MYC ON
- MYC OFF
- MYC BACK ON

**Lipid Composition**

**c-MYC Expression**

**Dissecting tissue**
**Extraction**
**HPLC-MS**

**CHEMICAL IMAGING**
Ideal to acquire simultaneously

**Tissue type**
**Disease Stage**
Previous studies show that DESI imaging is great for detecting lipids in tissues.

What type of information can we obtain using DESI imaging MS?
Liver: c-MYC Activated for 4 Months

Lipid Species Only Present in Normal Tissue

Tumors

‘Normal’ (Adjacent)
Images show that each species has a different intensity profile across the tissue.
Lipid Species Present in Both Tumor Regions

- m/z 282
- m/z 318
- m/z 772
- m/z 810
- m/z 563
- m/z 748
- m/z 820
- m/z 796
Lipid Species Present in Only One Tumor Tissue

Different genotypes?
Lipid Species in Wild Type Tissue

Down-regulated in tumors

Up-regulated in tumors

Optical Image
Comparison of Wild Type and Adjacent Tissues

Wild Type

Adjacent

Plotted on the same intensity scale

m/z 215  m/z 537  m/z 886
Plotted on the same intensity scale

**Wild Type**

**Adjacent**

- Cells in the adjacent tissue are affected by the tumors – i.e. not normal
- Species tumors release into the extracellular space?
Late Stage Tumor

Observe time course of each lipid
Identifying the detected species...
Just a reminder...
Fatty Acids

Wild Type
6 wks

Myc ON
4 mos

Myc OFF
6 mos

Myc ON
2 mos

Late Stage Tumor

Difference between wild type and adjacent tissue – Warburg effect?

18:1

\[-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}==\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-\text{COOH}\]
Monoacylglycerophosphoglycerols

Wild Type

6 wks

MYC ON

4 mos

MYC OFF

6 mos

MYC ON

2 mos

Late Stage Tumor

18:1
16:0
20:3
Diacylglycerophosphoglycerols

Wild Type
6 wks

Myc On
4 mos

Myc Off
6 mos

Myc On
2 mos

Late Stage Tumor

Different kinetics!

18:2/20:3
18:1/20:2
20:3/20:4
20:4/22:6
Diacylglycerophosphoinositols

Wild Type

MYC ON

Late Stage Tumor

Down-regulation in the entire tissue

MYC OFF

MYC ON

18:1/16:1
18:1/18:3
18:1/18:1 Diacylglycerophosphoinositol

Early stage lipid species!

Wild Type

6 wks

MYC ON

4 mos

MYC ON

6 mos

MYC OFF

2 mos

Late Stage Tumor
The people who make anything possible...

Fe and Ru CTH
Prof. Robert Waymouth
Dr. Antonio De Crisci
Kristen Brownell

C-H Amination
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Jennifer Roizen

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