Applications of Desorption Electrospray Ionization (DESI)

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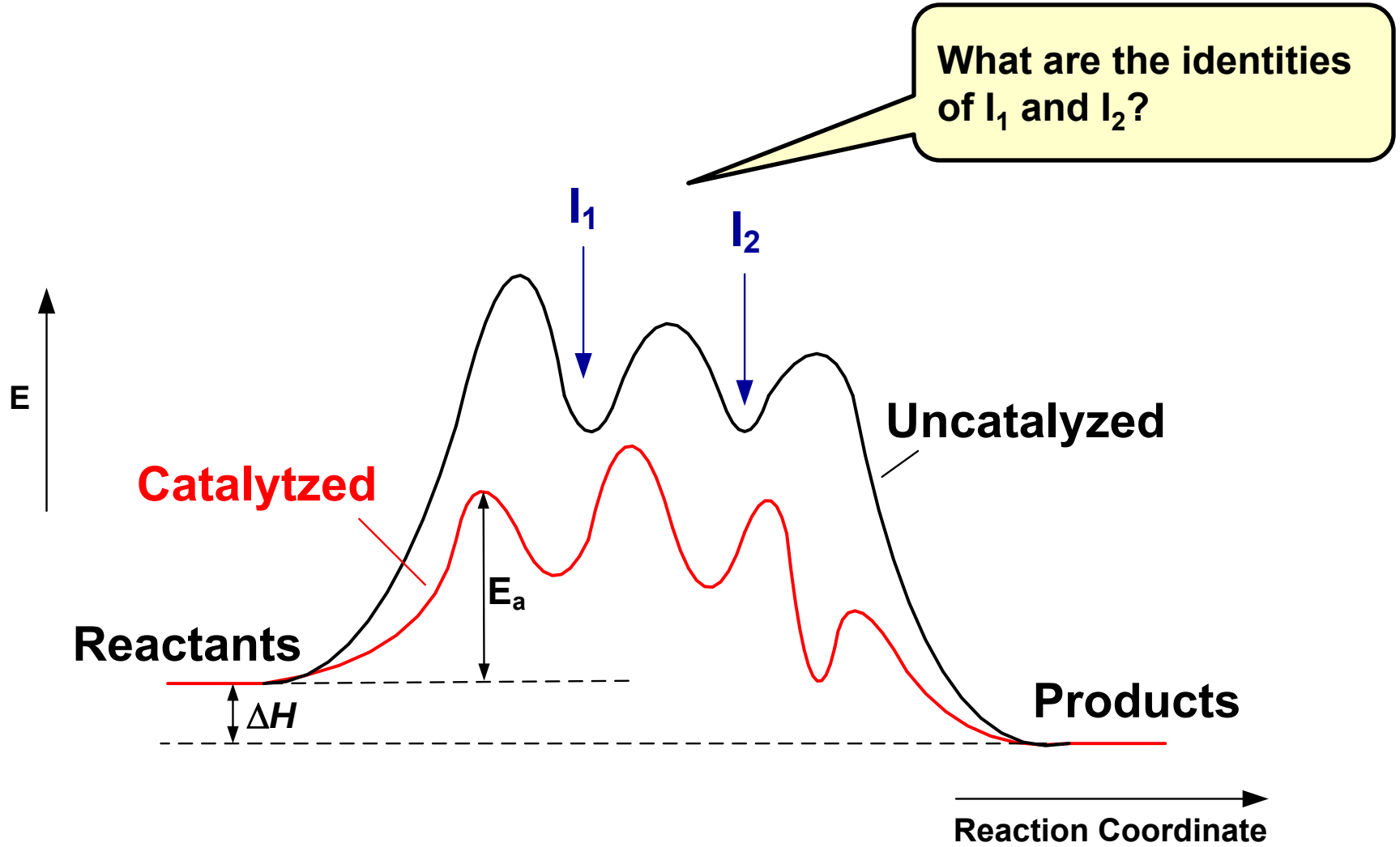
September 26, 2011

Presented at the 2011 Stanford Mass Spectrometry Users' Meeting



Identifying intermediates is critical for understanding chemical reactivity

CHALLENGES

- Short timescales
- Low concentrations
- Complex systems
- Side reactions

METHODS

- Optical Spectroscopy
- NMR
- Crystallography
- …

$$A + B \rightarrow \boxed{I_1 \rightarrow I_2} \rightarrow P$$

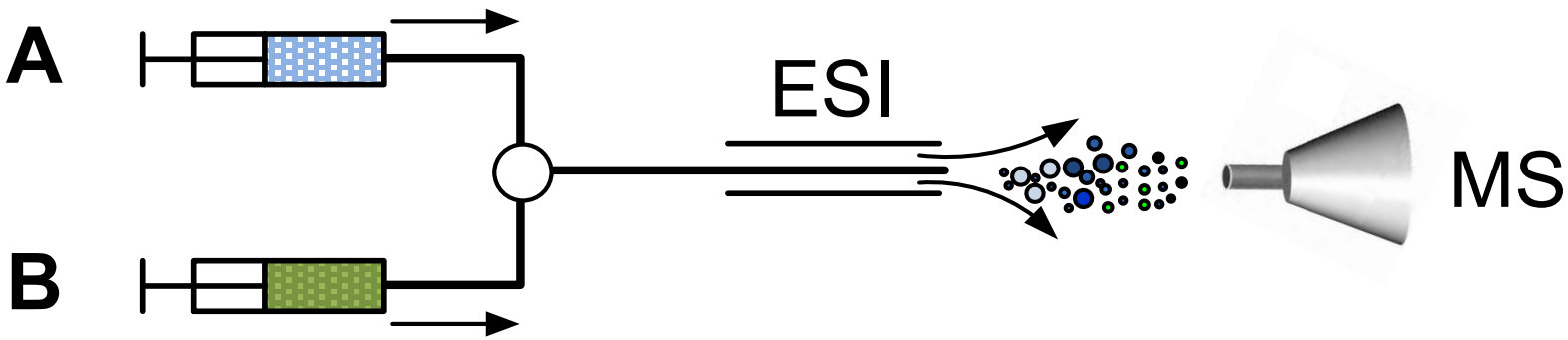
DISADVANTAGES OF THESE METHODS

- **Relatively long timescales**
- **Poor sensitivity**
- **Require relatively pure samples**

Mass spectrometry (MS) overcomes many of these limitations

Continuous-Flow

Reaction times of 5 ms



DISADVANTAGES OF THESE METHODS

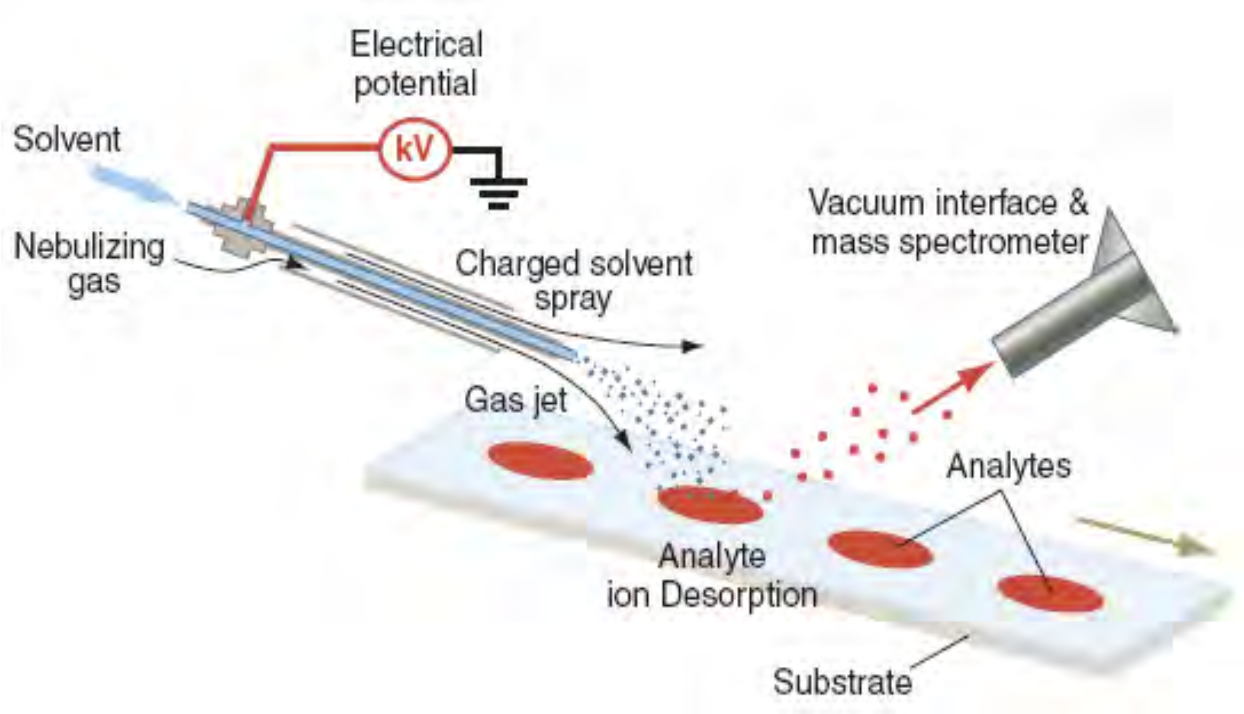
- **Not easily amenable to high-throughput**
- **Carry-over effects**

GOAL

Develop a simpler and cleaner approach for studying reaction mechanisms with millisecond time resolution

Desorption Electrospray Ionization (DESI)

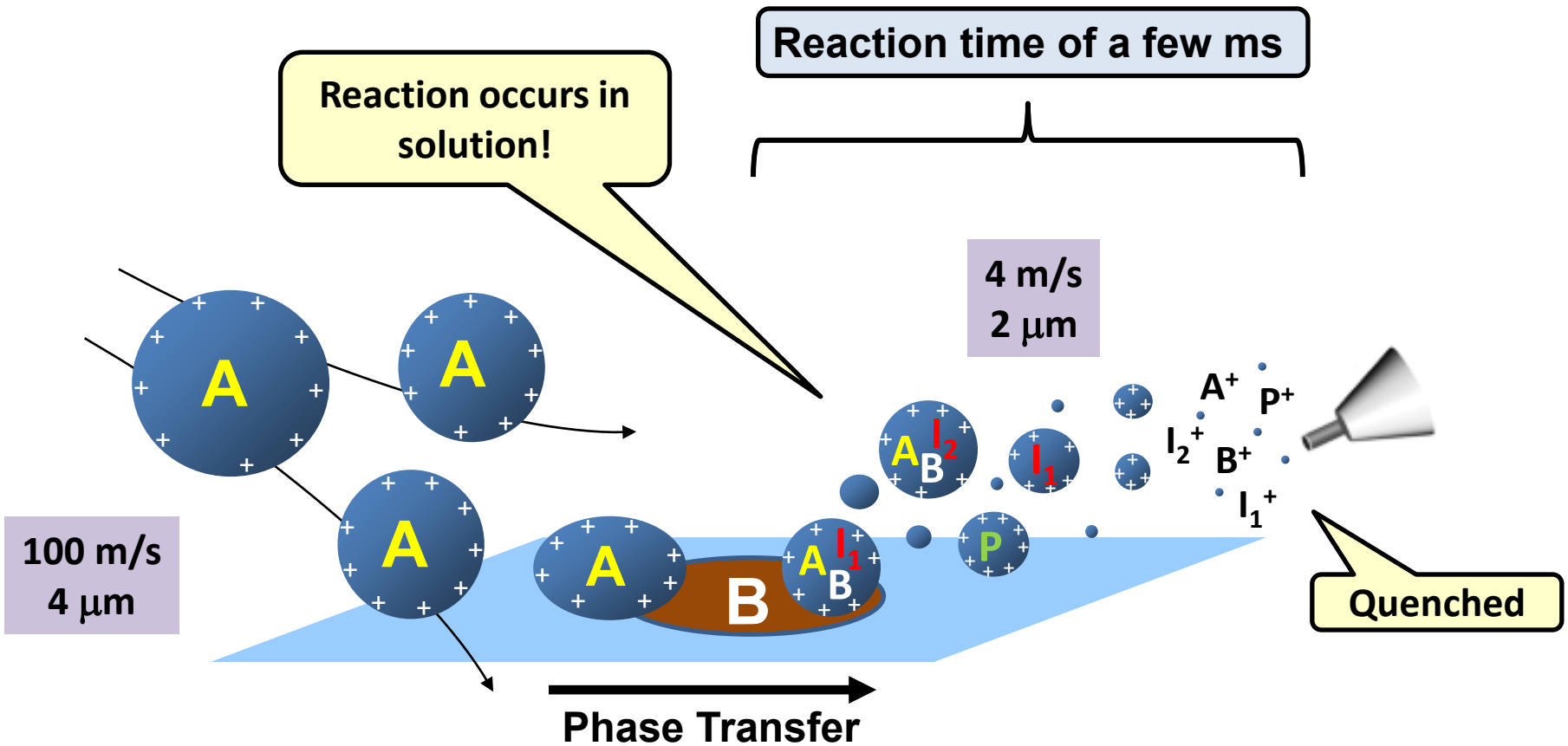
- **Ionization in open environment → Ambient MS**
- **No sample preparation**
- **High throughput**
- **Simple**



Z. W. Takats et al. *Science* **2004**, *306*, 471-473.
R. G. Cooks et al. *Science* **2006**, *311*, 1566-1570.

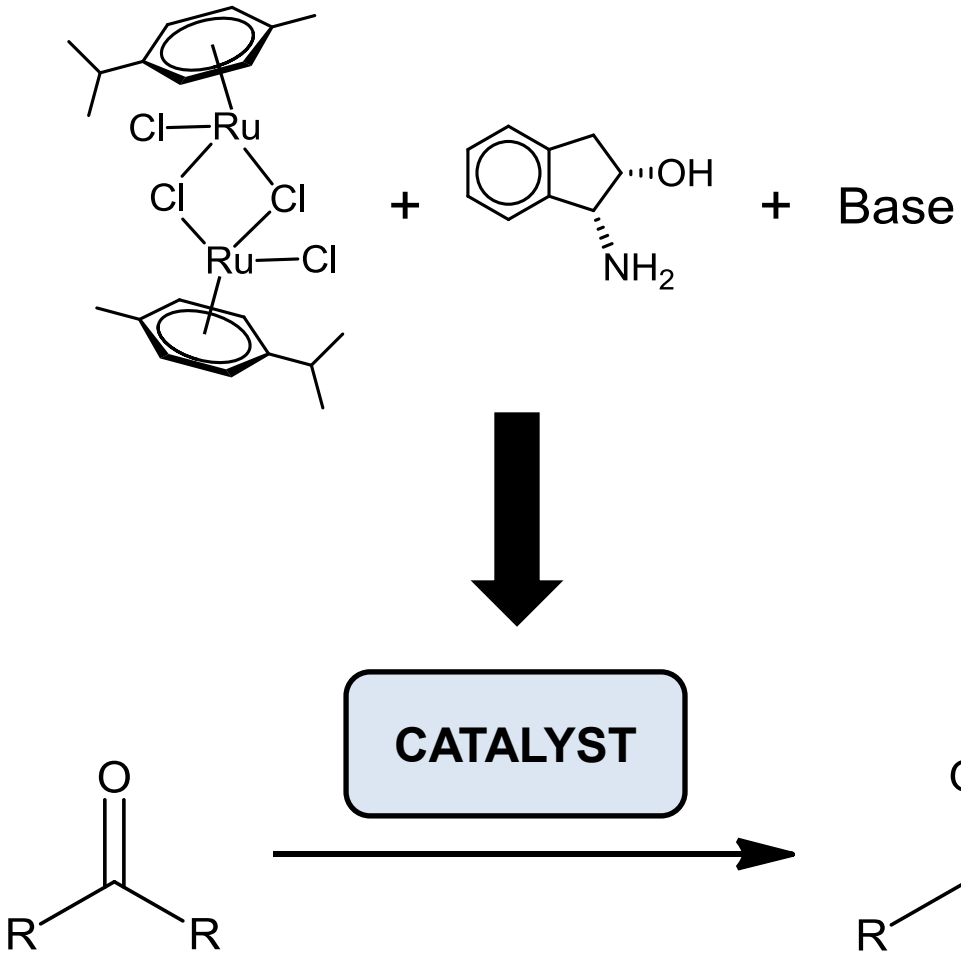
Using DESI to Access the Millisecond Timescale

$$A + B \rightarrow I_1 \rightarrow I_2 \rightarrow P$$



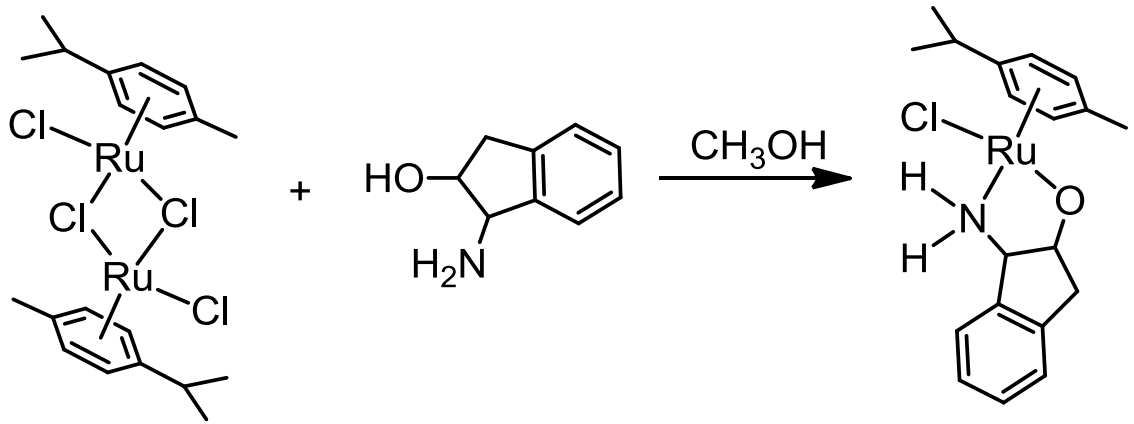
R. H. Perry et al. *Angew. Chem. Int. Ed.* **2011**, *50*, 250-254.

Ru (II)-Promoted Hydrogen Transfer



R. Noyori and S. Hashiguchi, *Acc. Chem. Res.* **1997**, *30*, 97-102.
M. Palmer et al. *J. Org. Chem.* **1997**, *62*, 5226-5228.

Ru Complex **β-Amino Alcohol** **Ru-Cl**

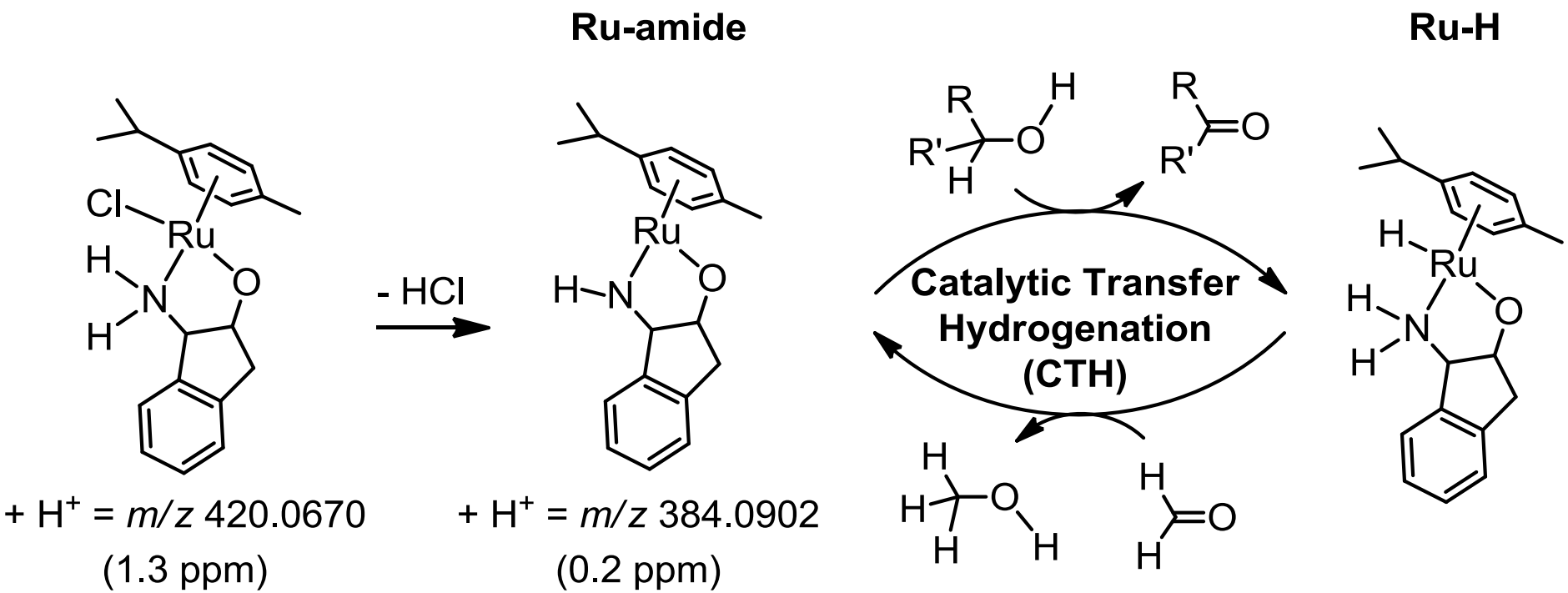


+ Na^+ = *m/z* 634.8921 (1.4 ppm)

+ H^+ = *m/z* 150.0913 (0.4 ppm)

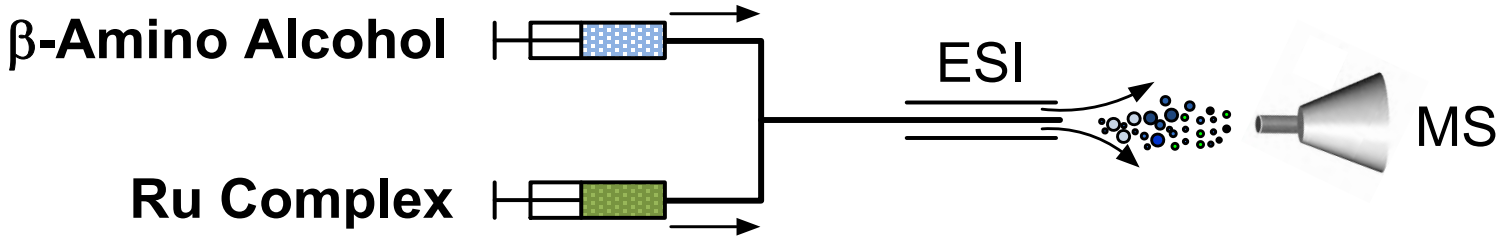
+ H^+ = *m/z* 420.0670 (1.3 ppm)

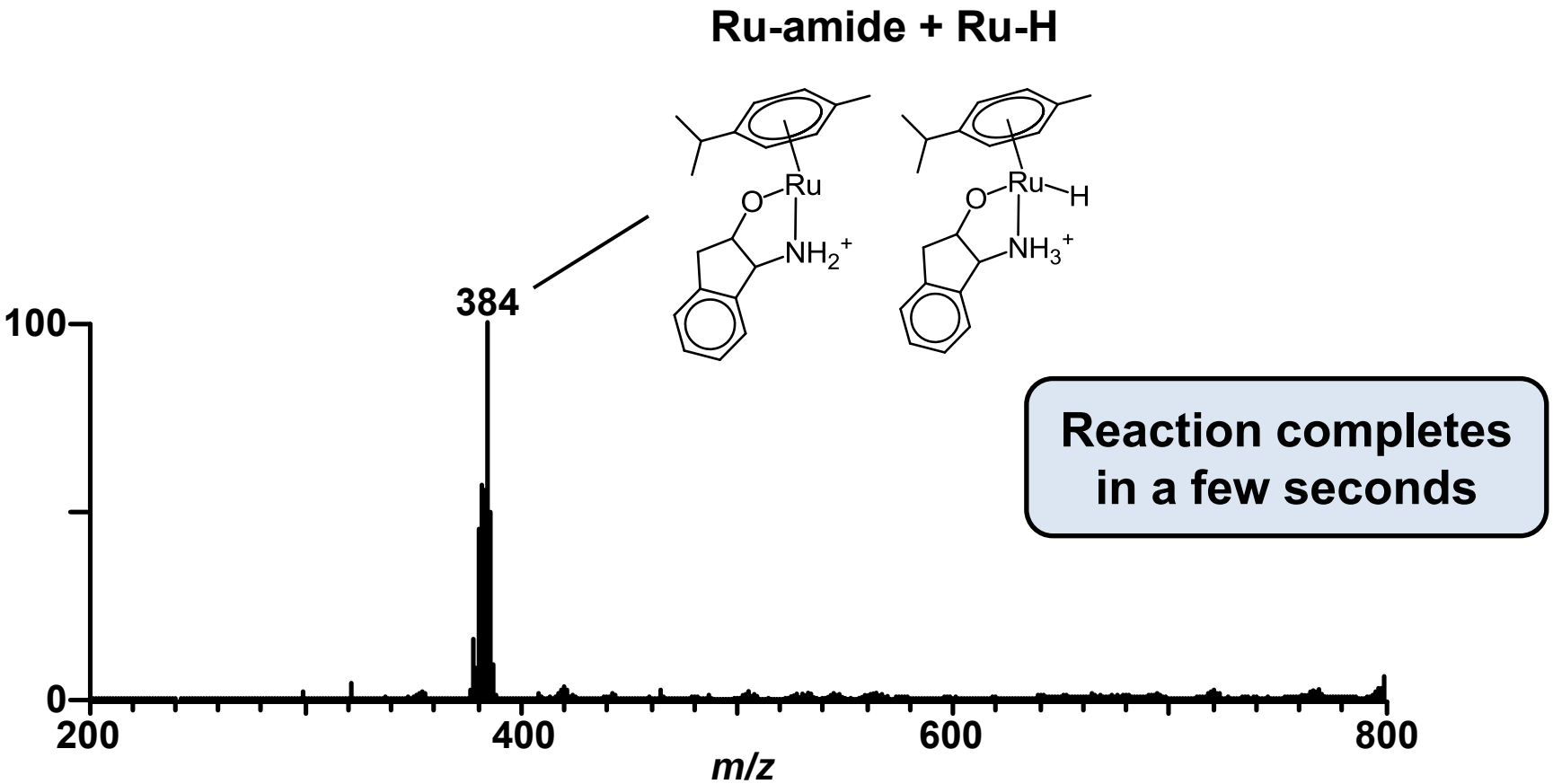
Kenny et al. *Chem. Commun.* **2000**, 99
Haack et al. *Angew. Chem. Int. Ed.* **1997**, *36*, 285



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

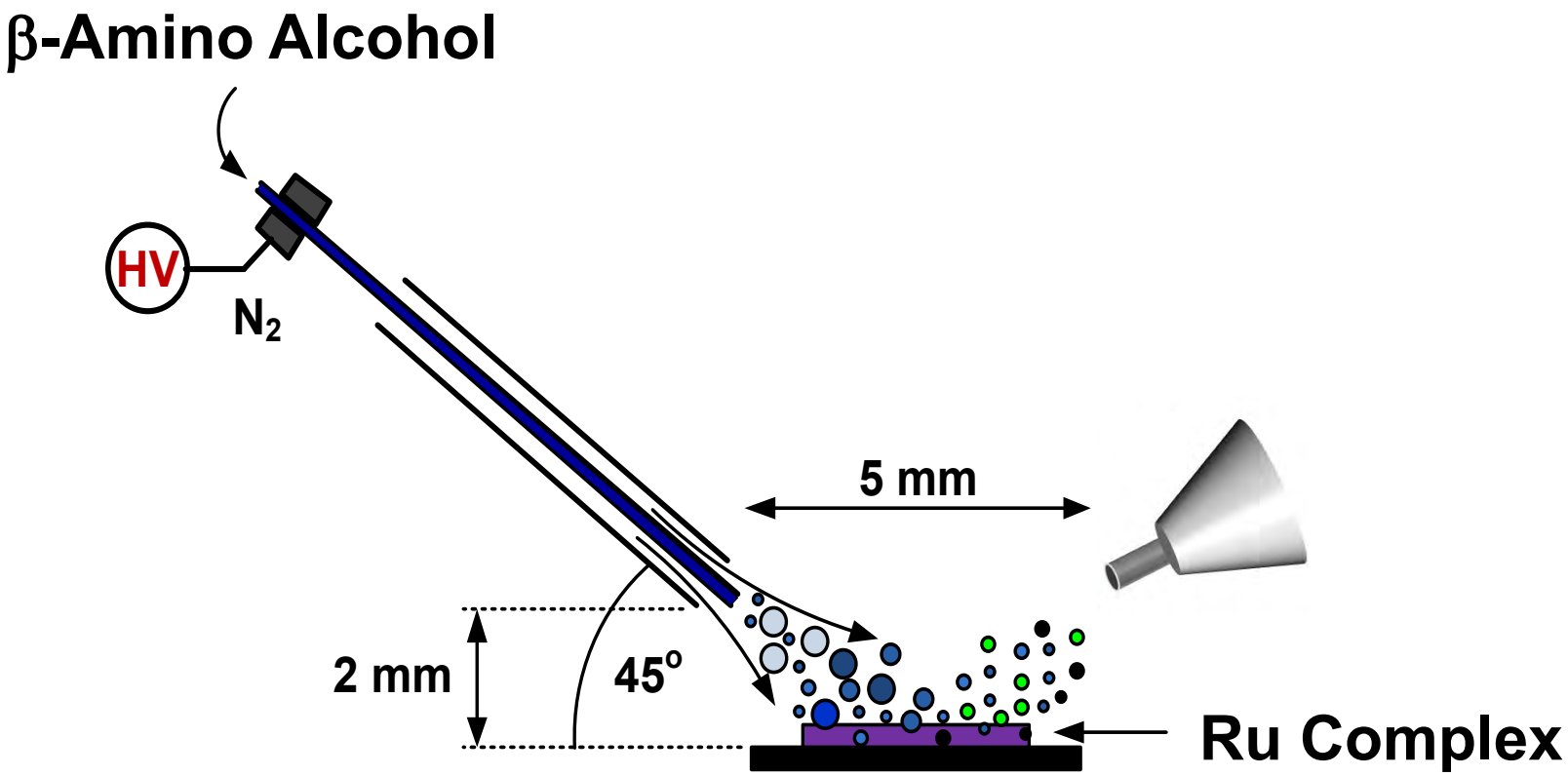
Kenny et al. *Chem. Commun.* **2000**, 99-100
Haack et al. *Angew. Chem. Int. Ed.* **1997**, *36*, 285





Perry et al. *Angew. Chem. Int. Ed*, **2011**, *50*, 250.

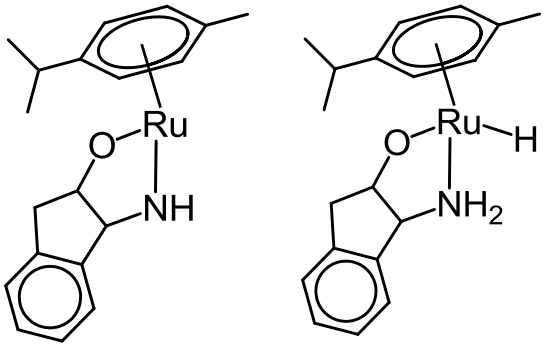
CTH by DESI



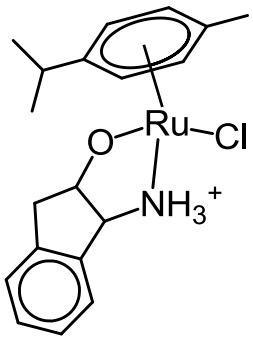
Perry et al. *Angew. Chem. Int. Ed*, **2011**, *50*, 250.

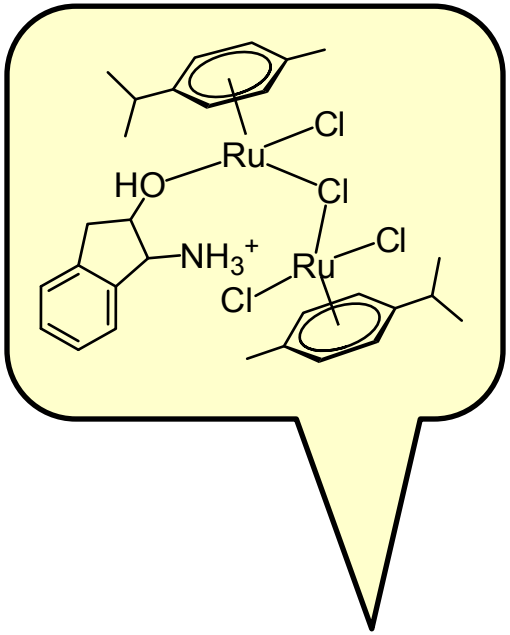
Ru-amide + Ru-H

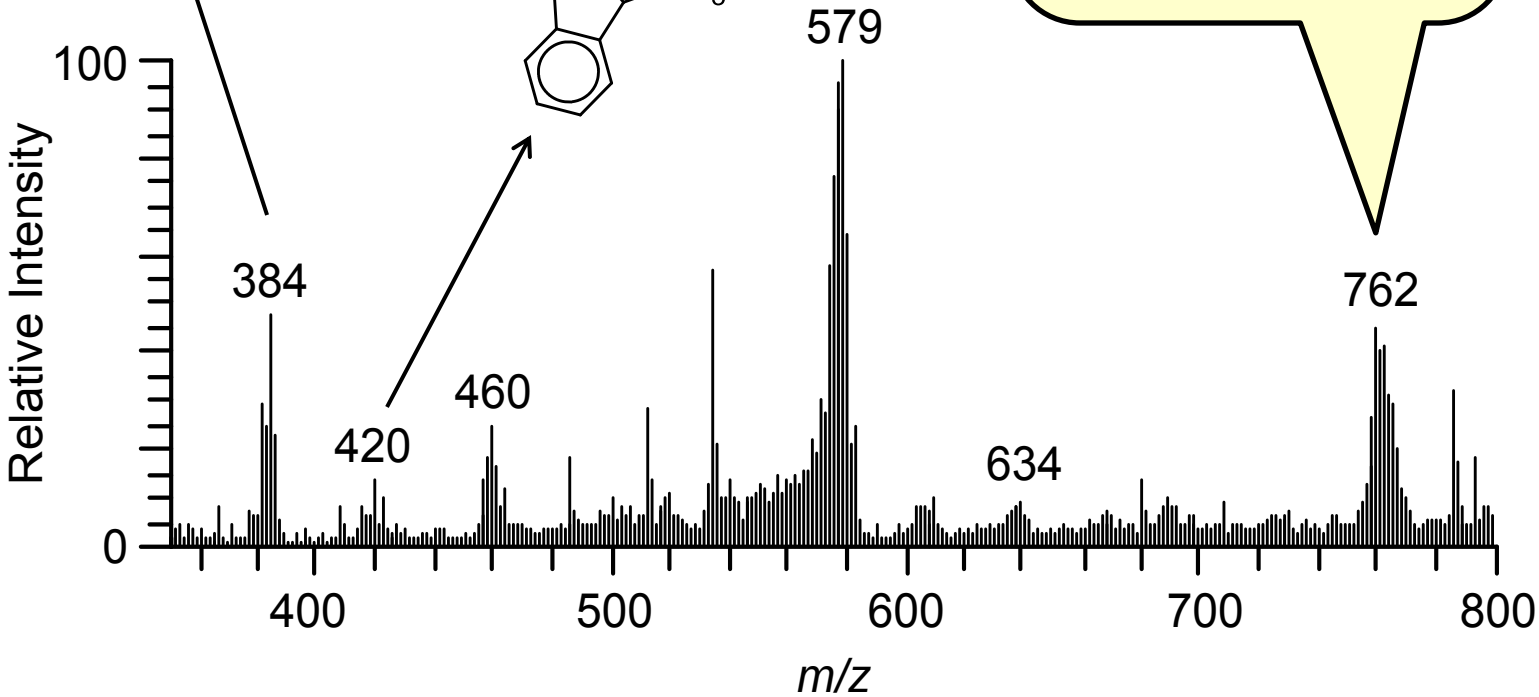
- **First intermediate of the reaction!!**



Ru-Cl



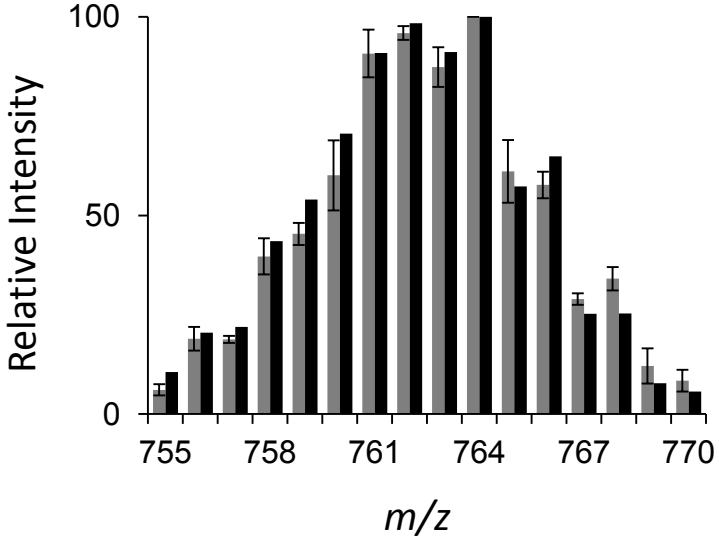




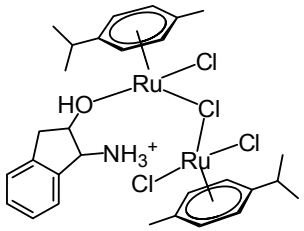
Perry et al. *Angew. Chem. Int. Ed*, **2011**, *50*, 250.

Confirming Peak Assignments

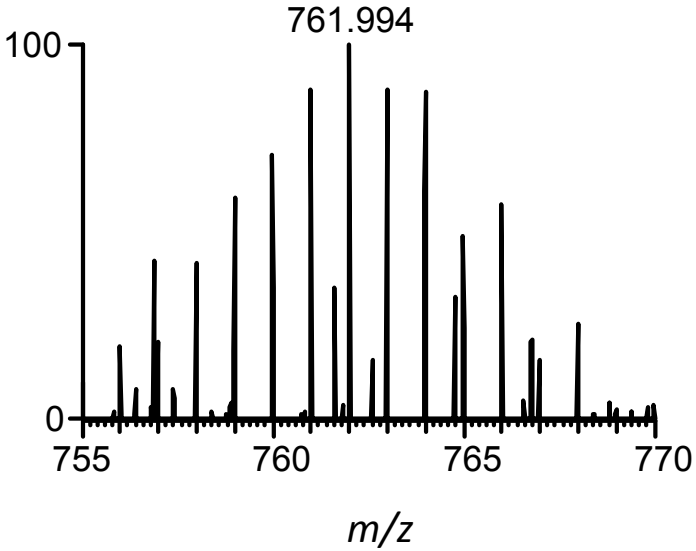
Isotope Ratio
%Error < 5%



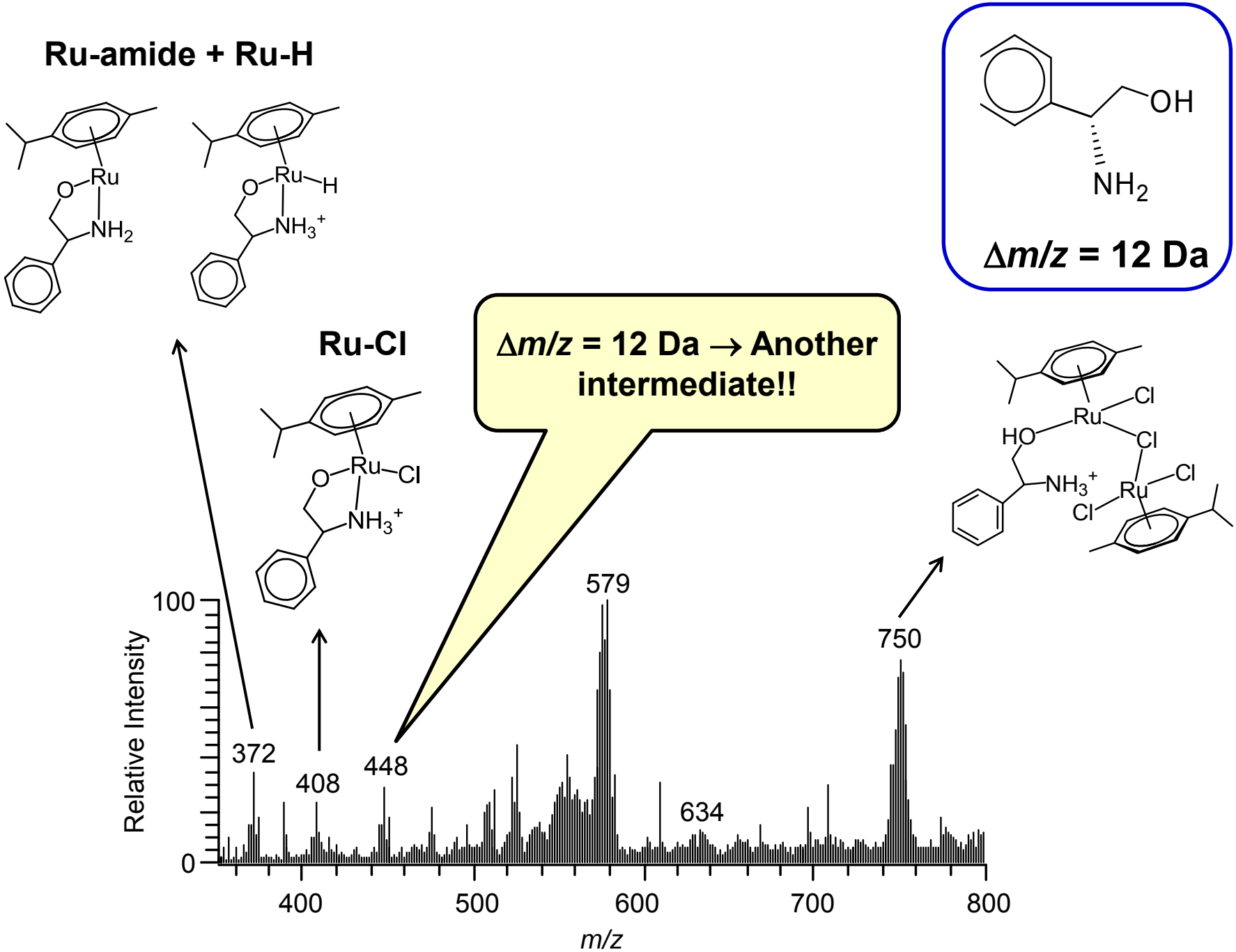
Black: Calculated
Grey: Experimental



Orbitrap Mass Spectra
$m/\Delta m$ = 80,000
Mass accuracy = 0.3 ppm



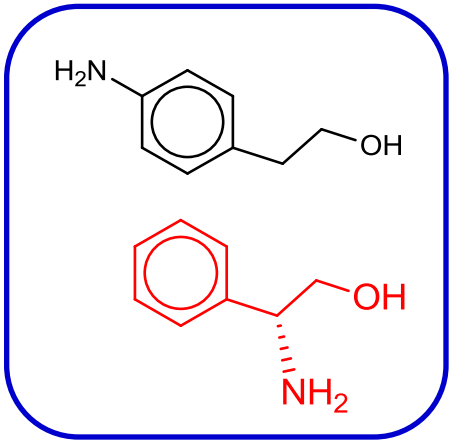
Perry et al. *Angew. Chem. Int. Ed*, **2011**, *50*, 250
Perry et al. *Angew. Chem. Int. Ed.* **2011**, in preparation

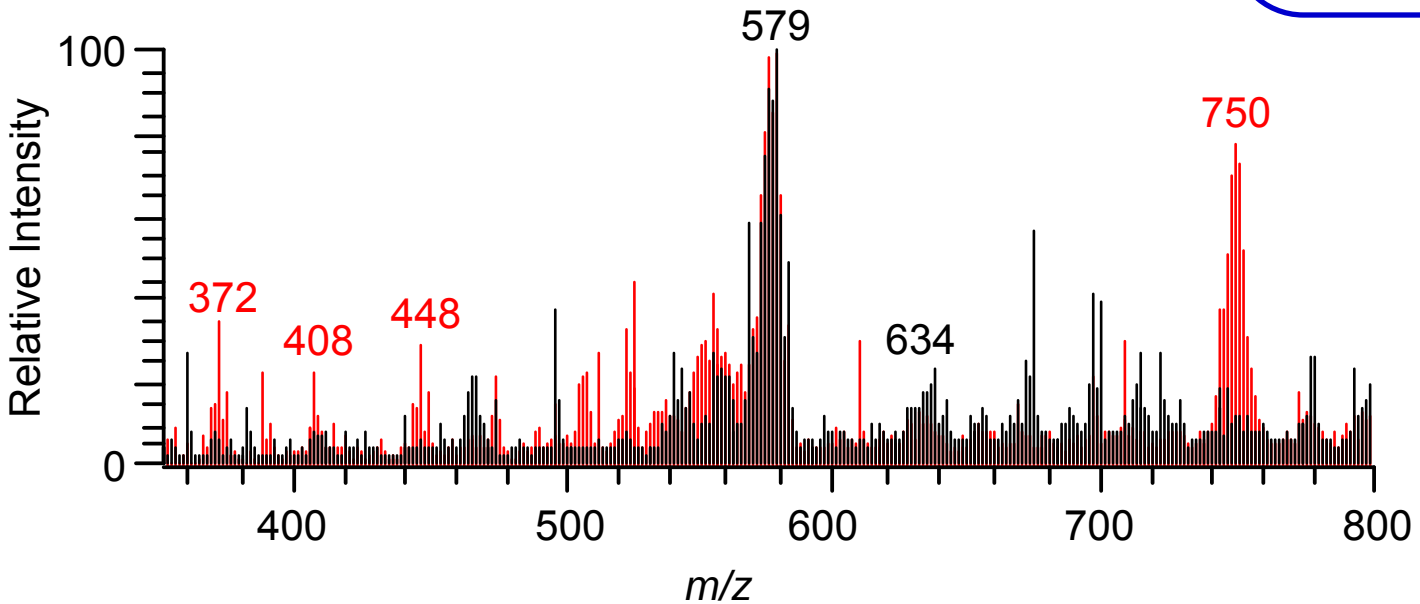


Perry et al. *Angew. Chem. Int. Ed*, **2011**, *50*, 250.

Relative –NH_2 and –OH positions are necessary for reactivity

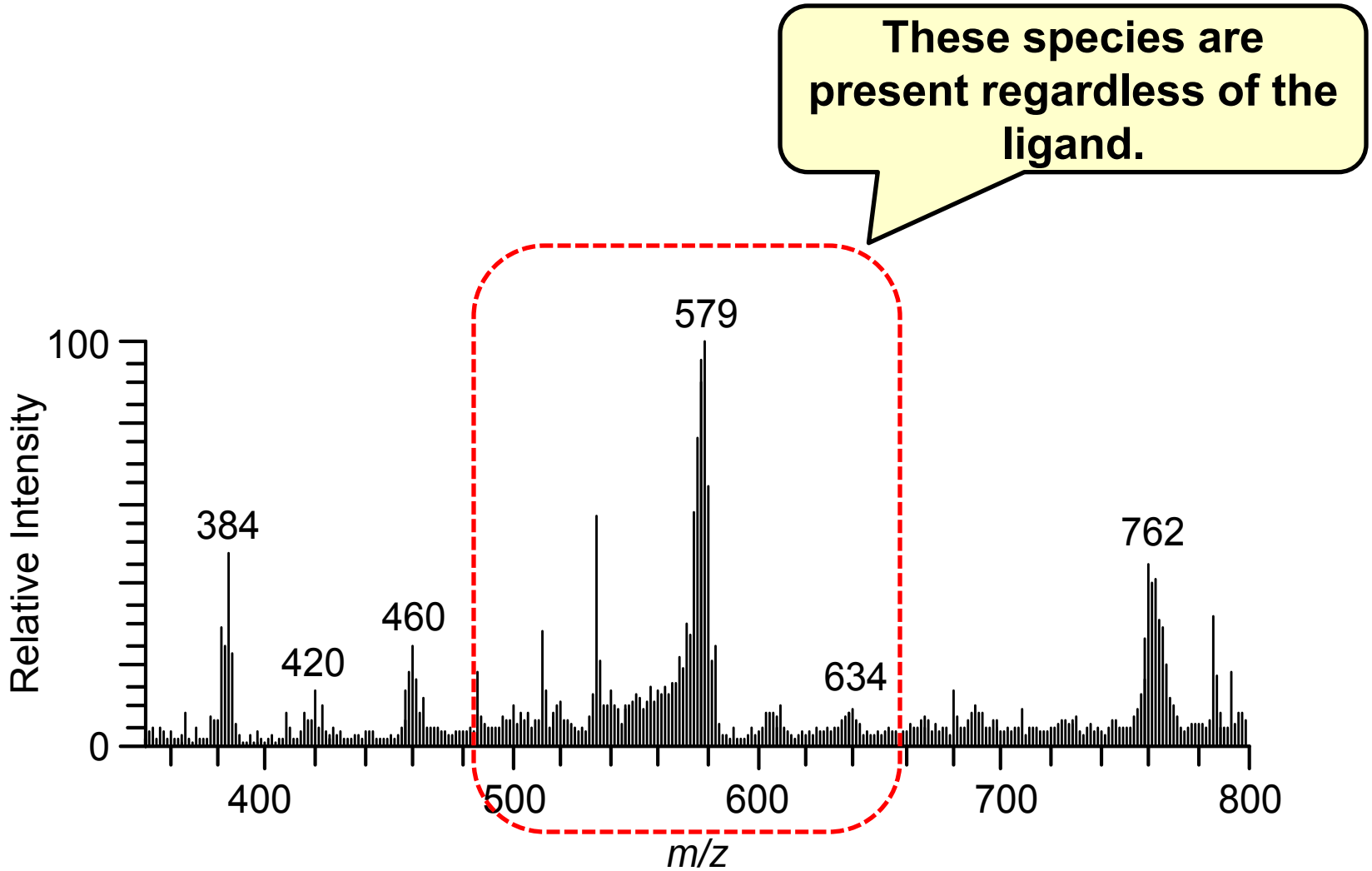
No CTH intermediates observed when NH_2 is farther away from –OH





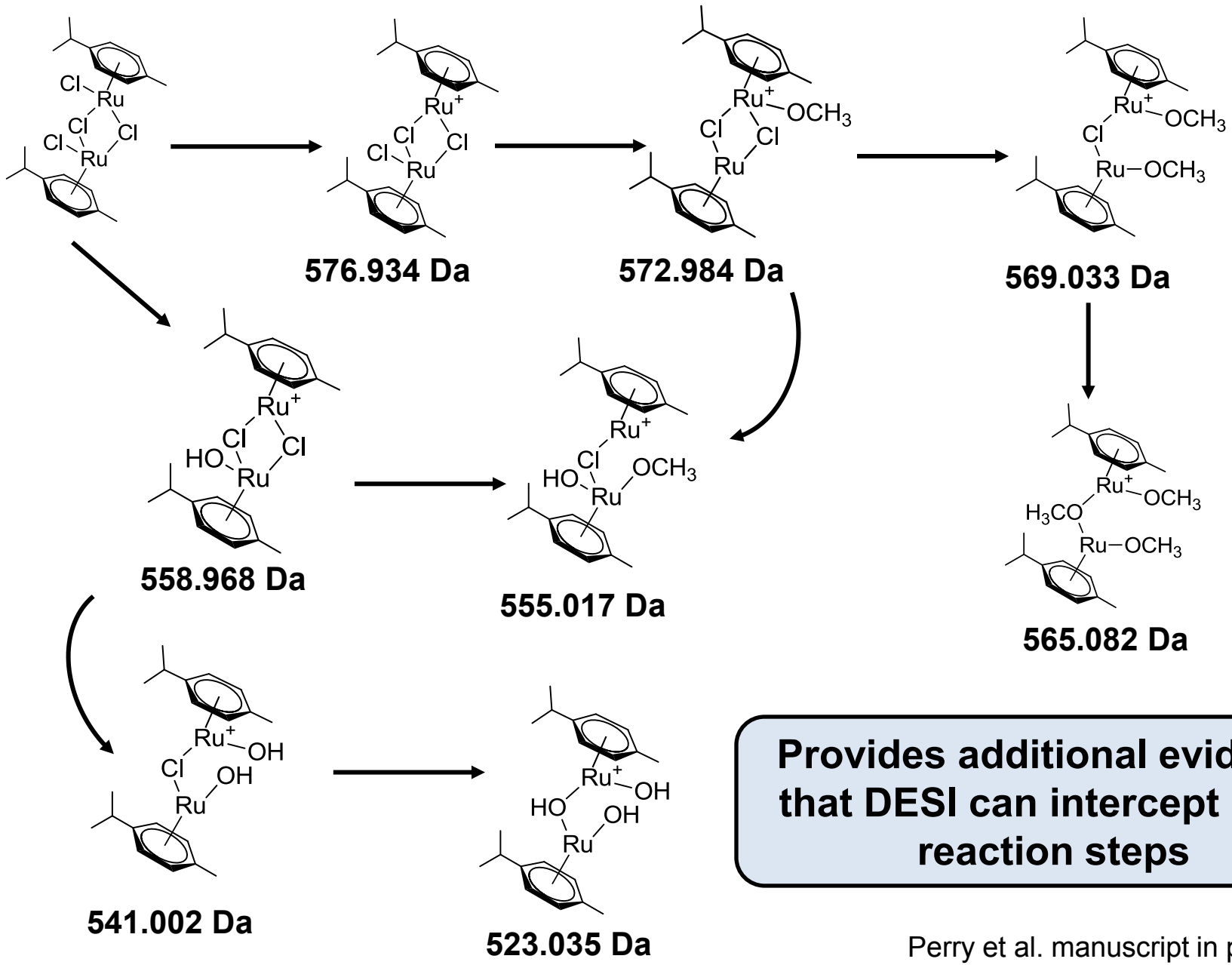
- **Detected species are relevant to the CTH reaction**
- **DESI can intercept intermediates on short timescales!!**

Perry et al. *Angew. Chem. Int. Ed*, **2011**, *50*, 250.



Perry et al. *Angew. Chem. Int. Ed*, **2011**, *50*, 250.

Intermediates Formed from Reaction with the Solvent



Provides additional evidence that DESI can intercept rapid reaction steps

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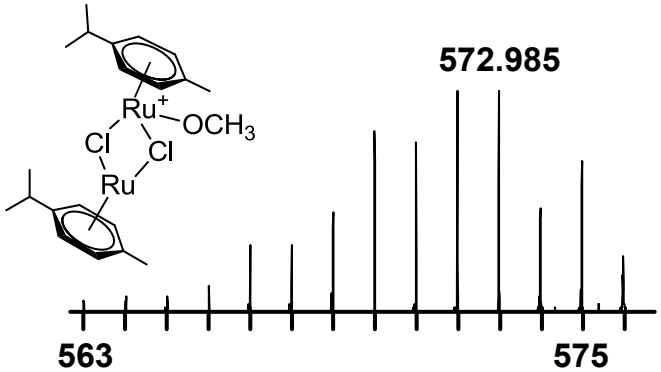
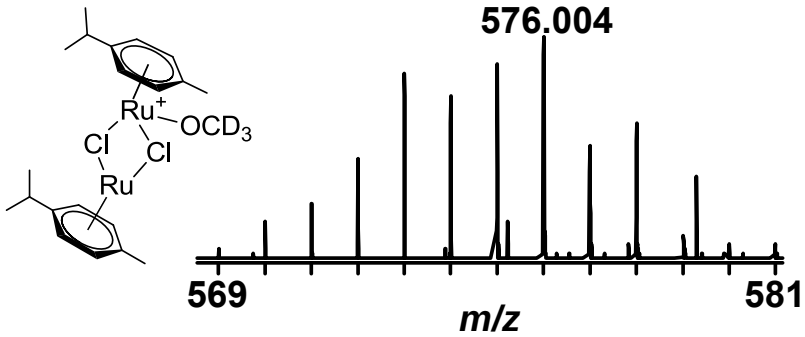
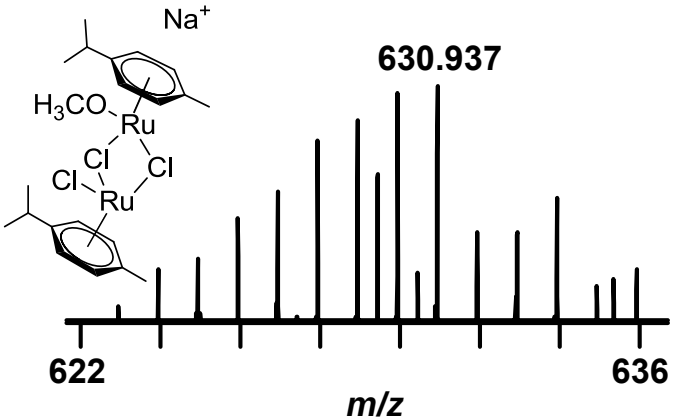
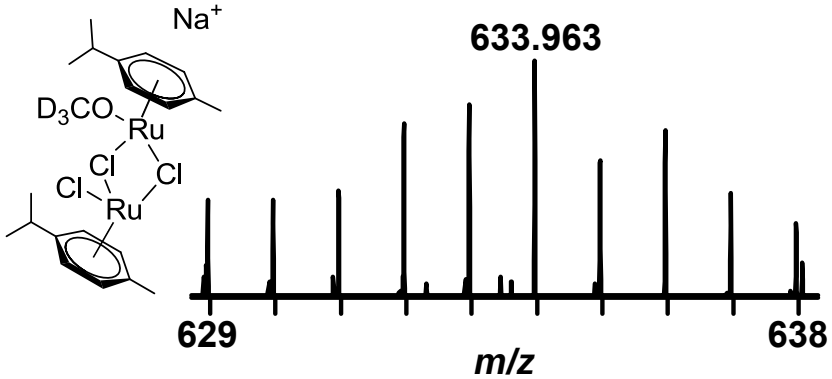
Confirmation of the intermediates formed with methanol





~3 Da Shift





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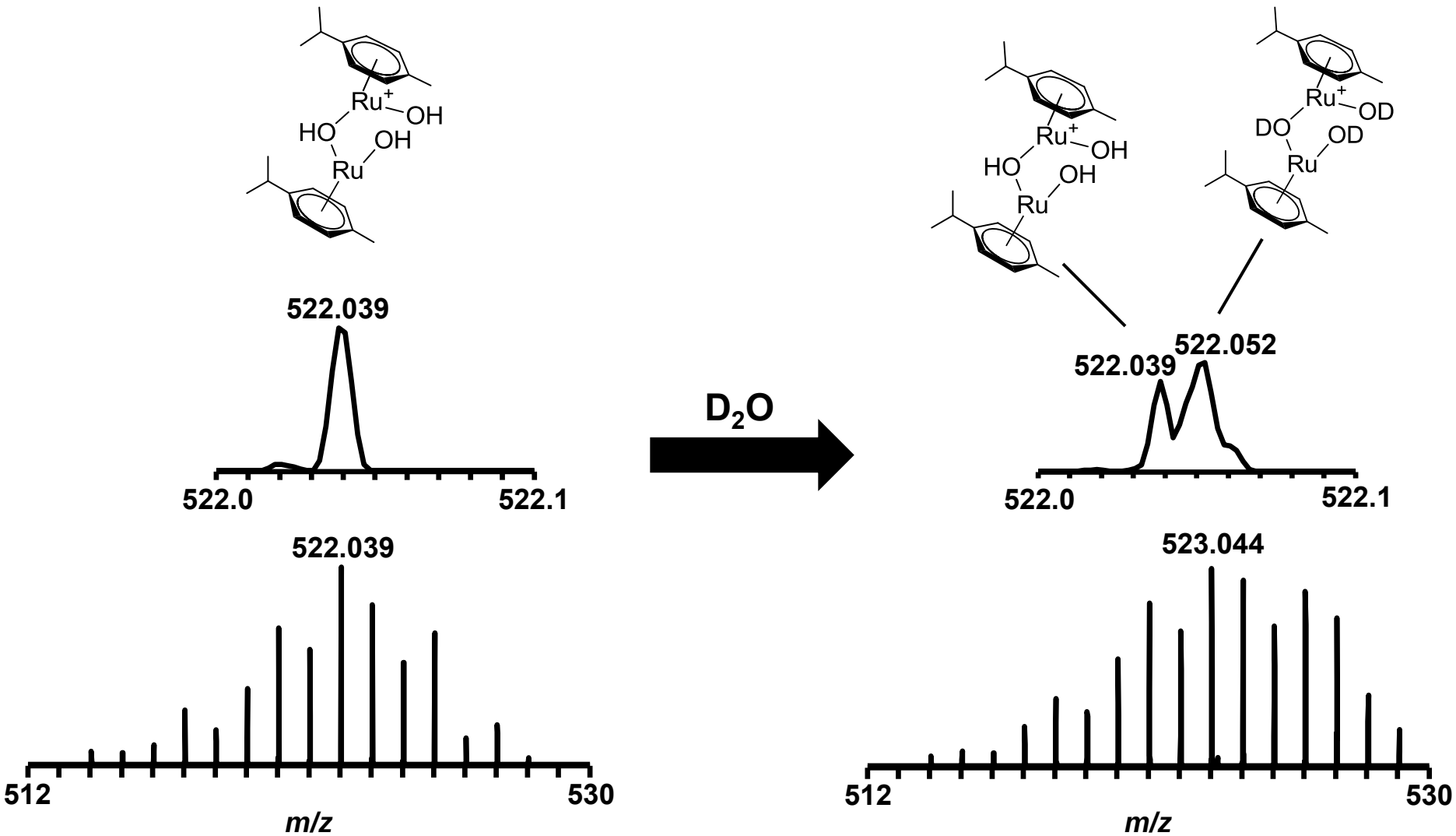
Confirmation of the intermediates formed with H_2O



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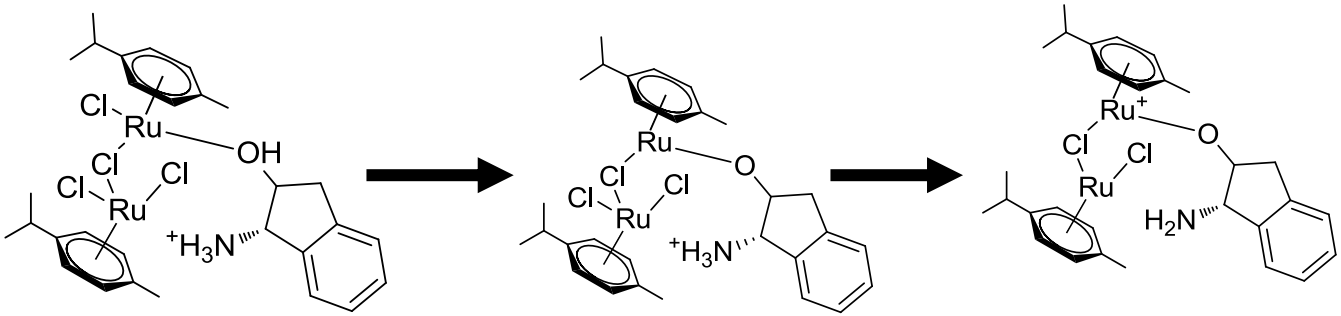
Summary

MILLISECONDS



761.995 Da (0.2 ppm) → **726.018 Da (0.7 ppm)** → **690.041 Da (0.4 ppm)**

- **Indicates an associative mechanism**
- **Observe step-wise breakdown of the dimer**
- **Intermediates with CH_3OH and H_2O observed**
- **Detected species agree with proposed catalytic mechanisms**

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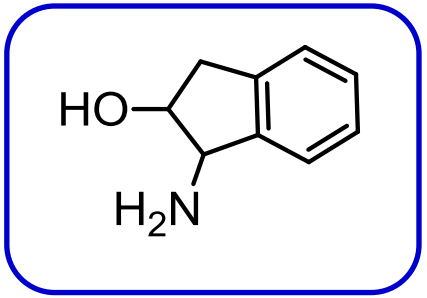
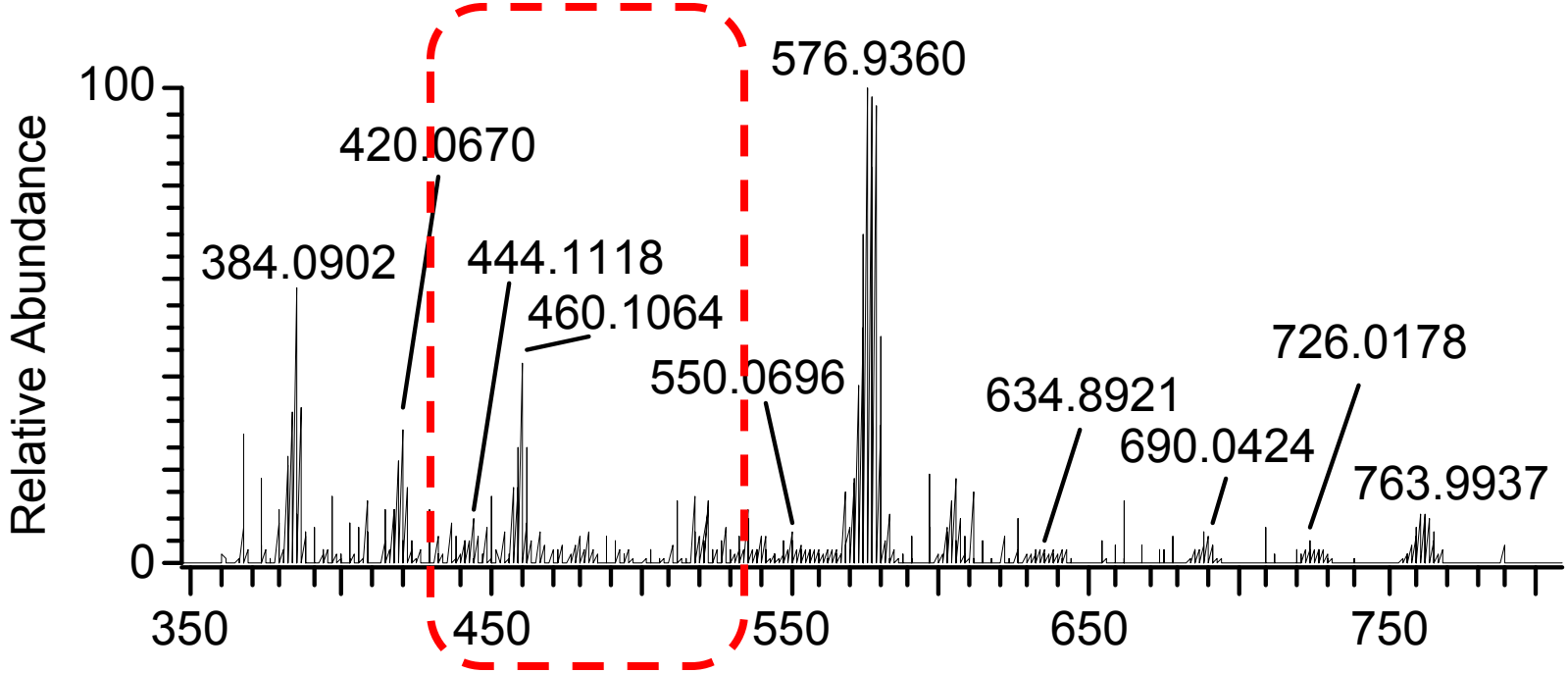
What can we learn about transfer hydrogenation catalysis?

What are the identities of *m/z* 444 and *m/z* 460?





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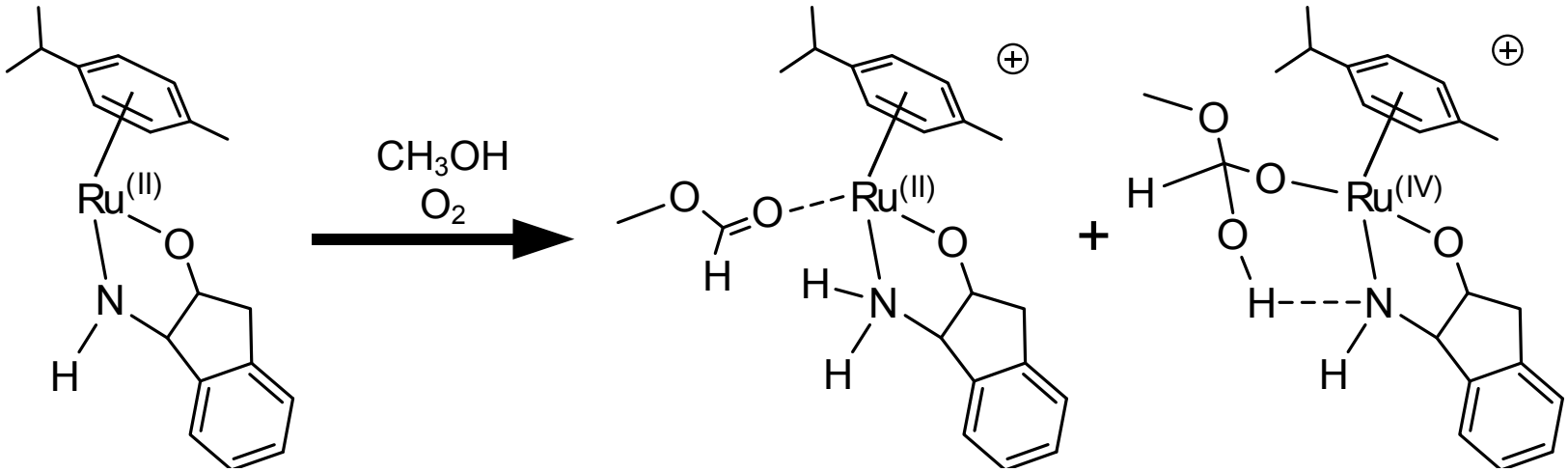
Ru-Methyl Formate Intermediates



m/z 444.1118
(1.1 ppm)

m/z 460.1064
(0.3 ppm)

- **Oxidative dehydrogenation of methanol to generate methyl formate**
- **Adventitious O_2 oxidizes Ru (II) to Ru (IV)**

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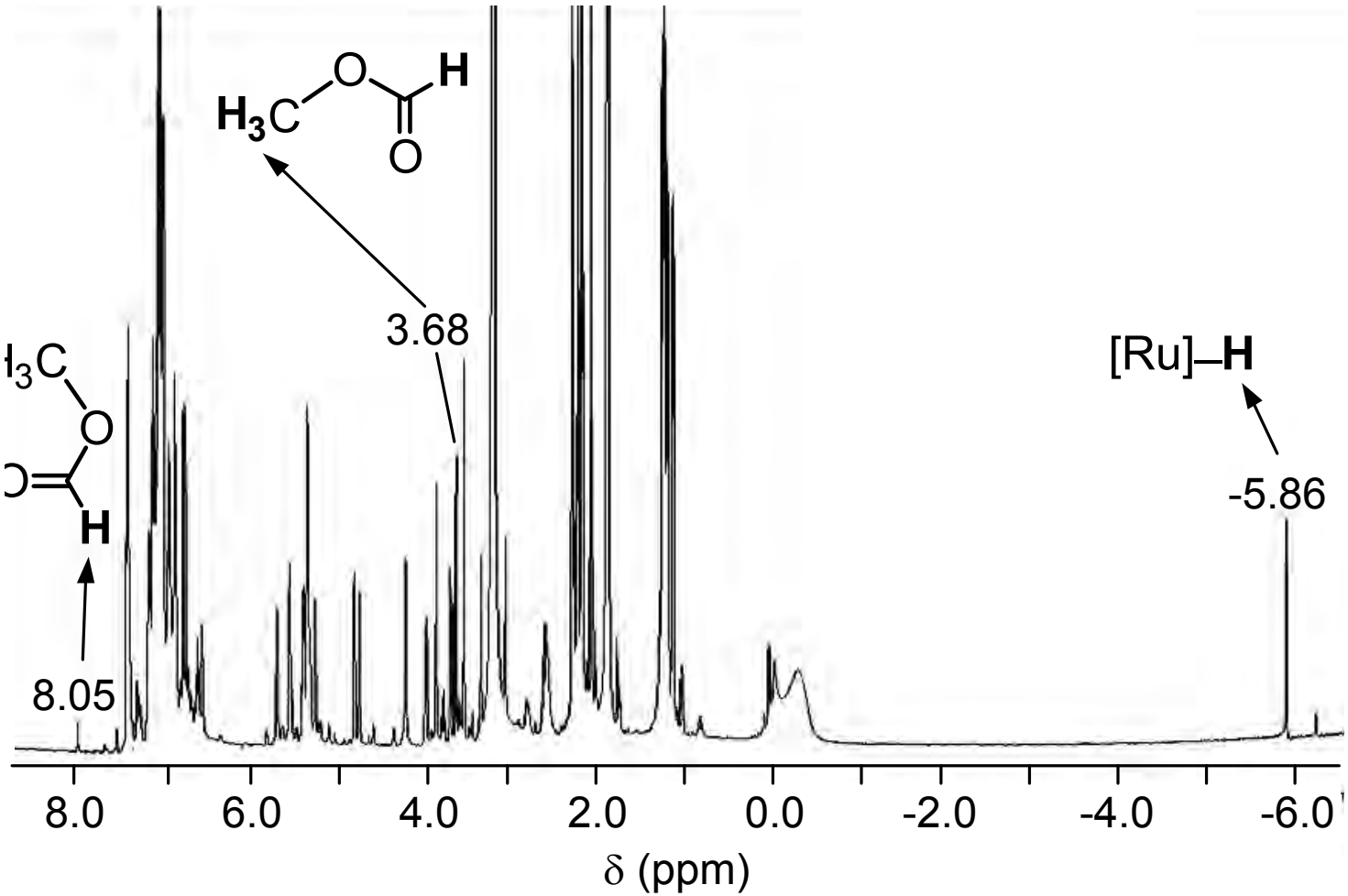
NMR



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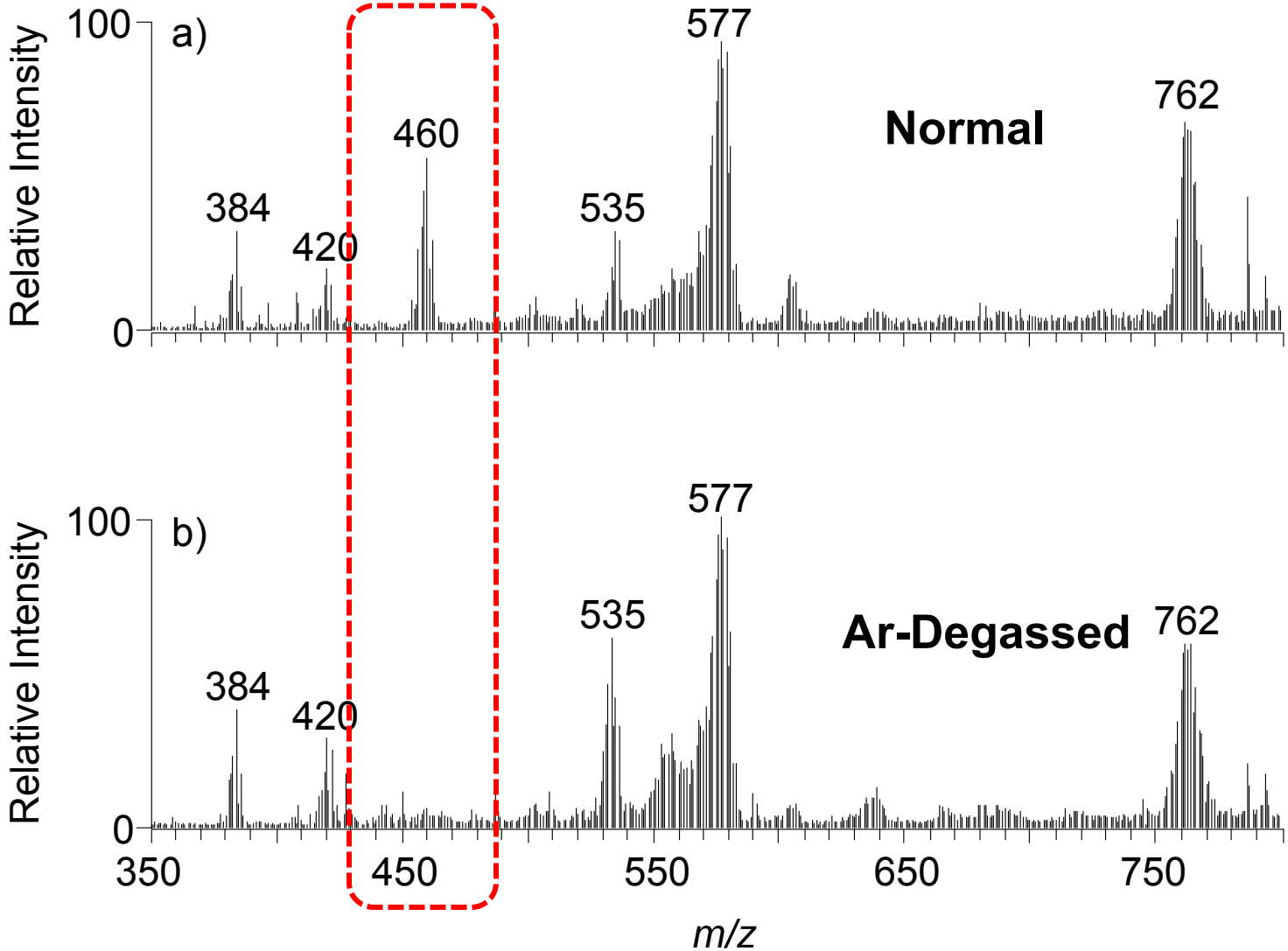
Oxidation of Ru (II) to Ru (IV) by Adventitious O_2



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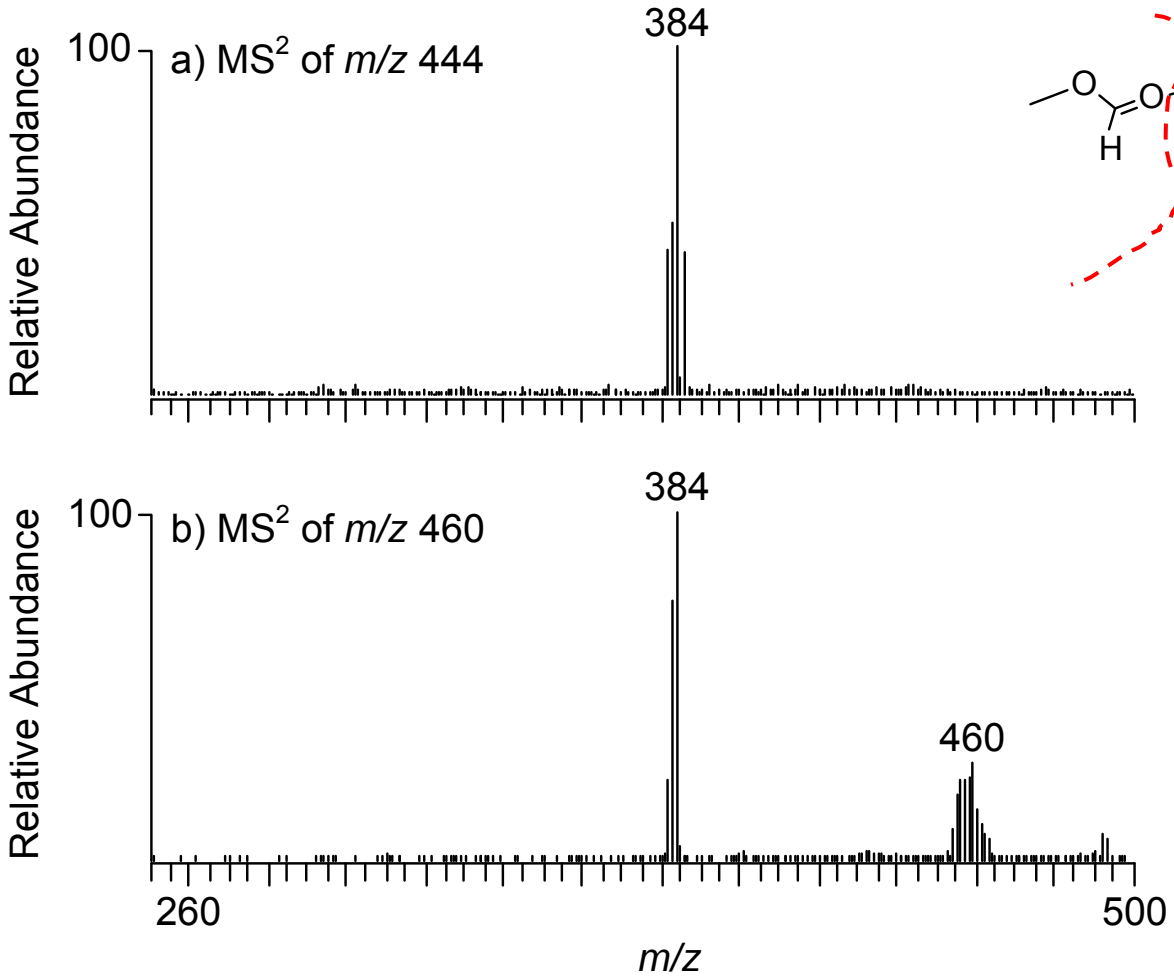
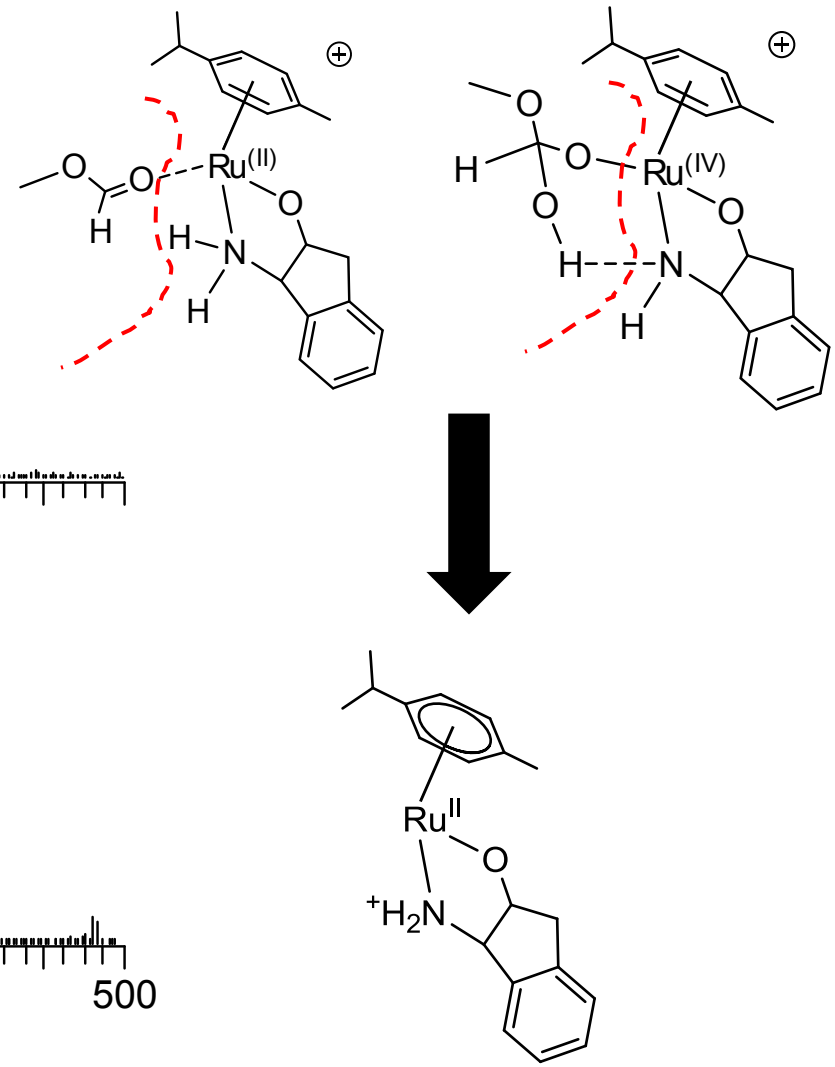
MS/MS

Normalized Collision Energy = 40%





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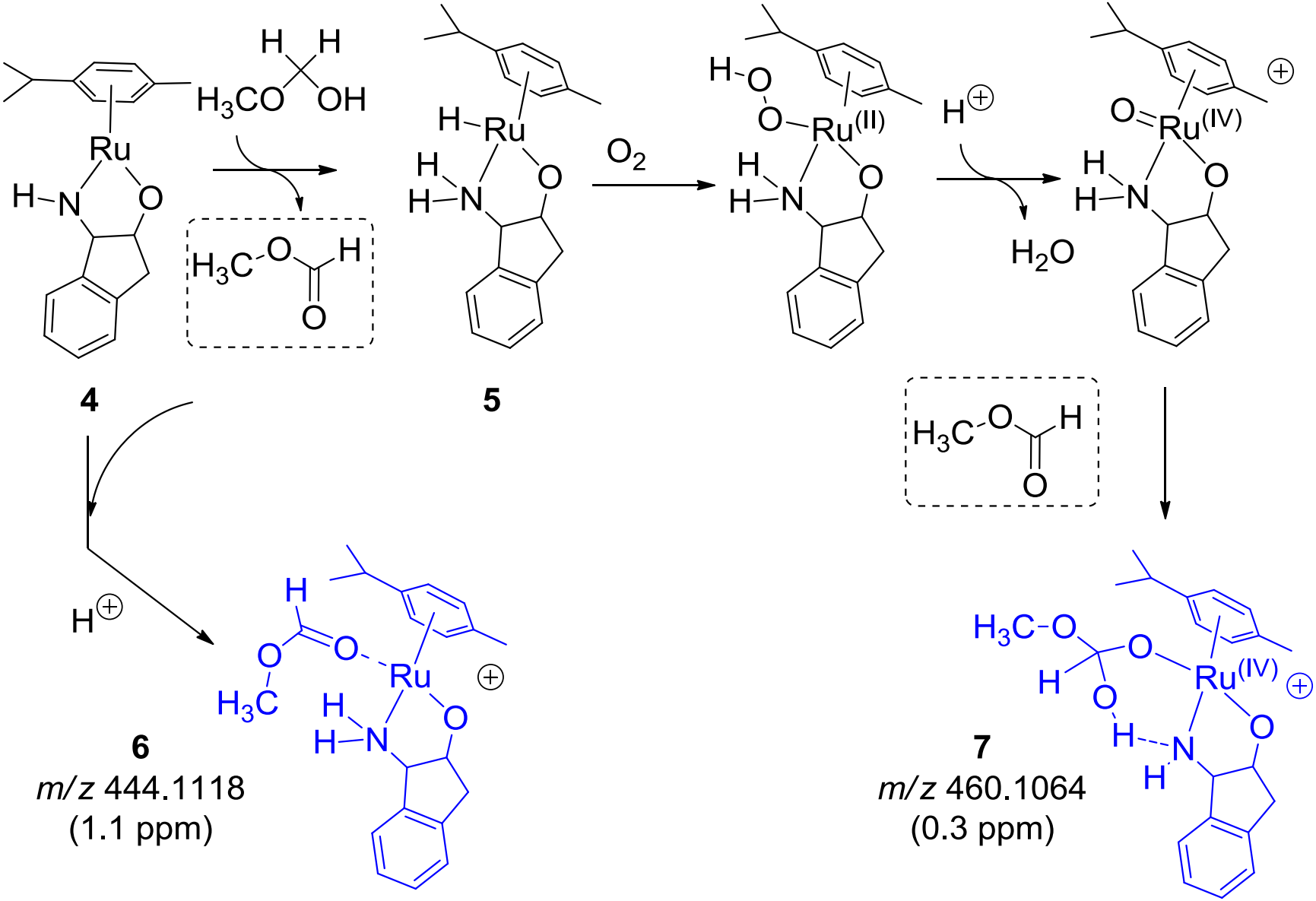
Putting it all together…



Perry et al. manuscript in preparation

What we discovered…

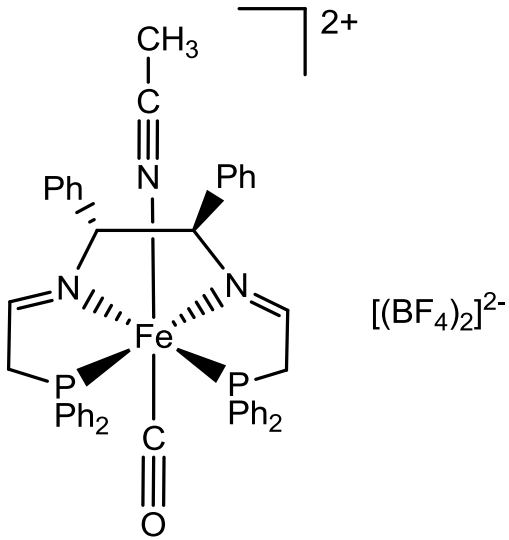
- **DESI can intercept intermediates on short timescales (millisecond regime)**
- **Detected new Ru (II) reaction intermediates in transfer hydrogenations catalyzed by (β-amino alcohol)(arene)Ru complexes**
- **Detected intermediates formed in the reaction with CH_3OH and H_2O**
- **Detected a Ru-methyl formate species that suggests formation of methyl formate in CTH**

Moving forward…

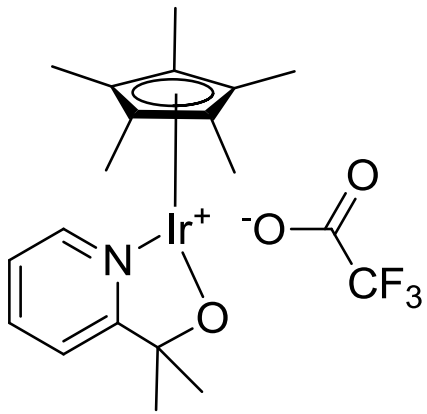
Combining the timescales of DESI and ESI provides a more complete picture of reaction pathways

Other Catalytic Reactions

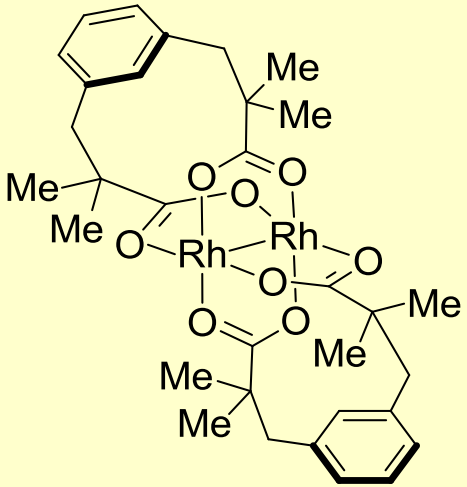
Transfer Hydrogenation



Water Oxidation

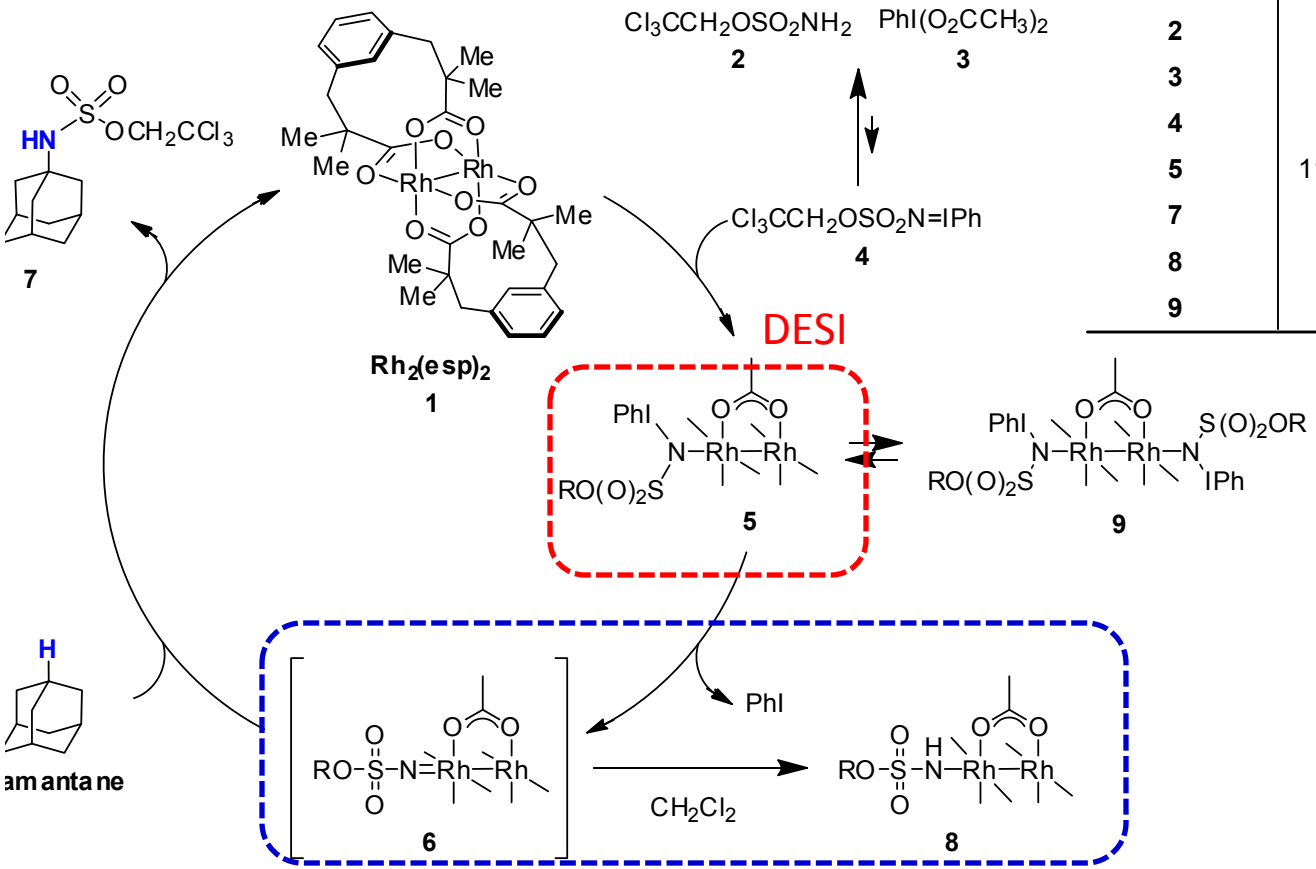


C-H Amination



Many of the proposed intermediates of these catalytic systems short-lived and have not been directly observed

C-H Amination



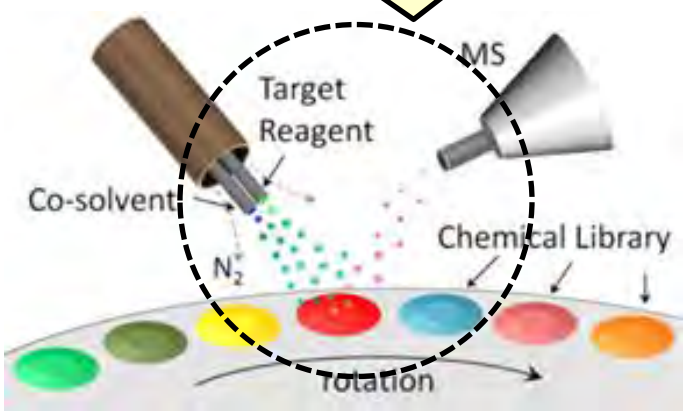
Compound	$[M]^{+\cdot}$ *m/z* (ppm)	$[M+Na]^+$ *m/z* (ppm)
1	758.0808 (2.6)	781.0724 (0.1)
2		249.8863 (2.6)
3		344.9591 (1.1)
4		451.8144 (1.0)
5	1186.9057 (2.4)	
7		383.9959 (1.6)
8		1006.9618 (0.7)
9		1615.7342 (0.3)

- **Nitrenoid reacts through an H-atom abstraction pathway**

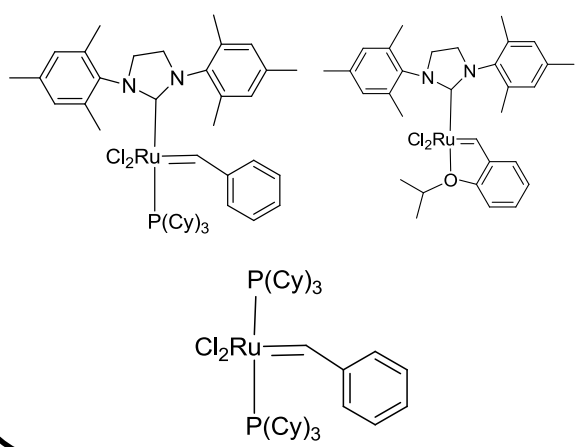
Cahill, Perry, Roizen, Davis, Du Bois, and Zare. manuscript in preparation.

High-Throughput Screening of Catalytic Mechanisms

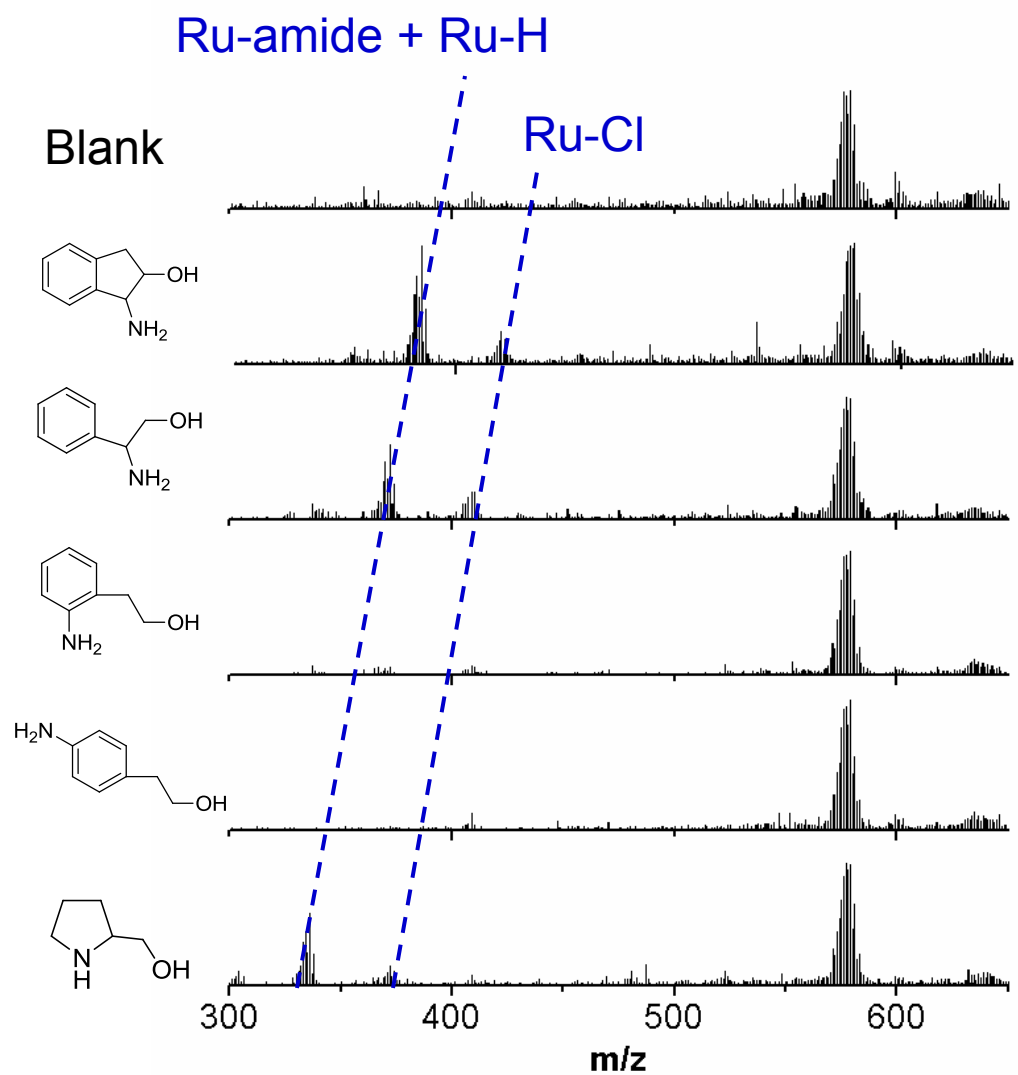
Air-sensitive reactions



Catalyst Screening



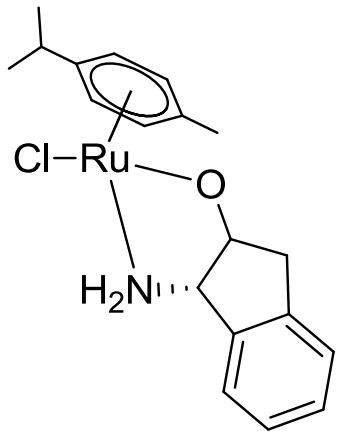
Ligand Screening

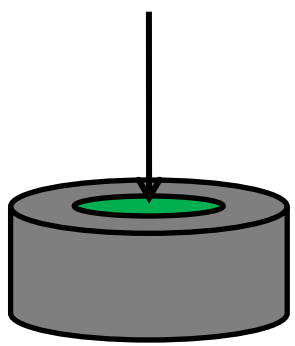


Perry et al. manuscript in preparation.

DESI-MS and Electrocatalysis

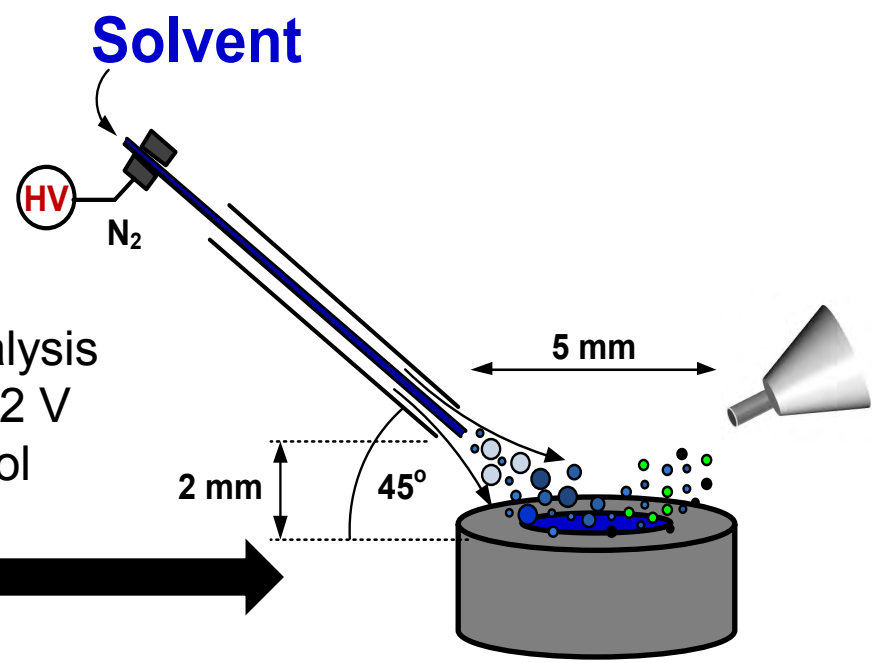
- This electrocatalytic reaction results in methanol oxidation
- Transfer hydrogenation catalysts can store and release energy for the electron economy





Graphite electrode

Electrocatalysis
1.1 V – 1.2 V
Methanol
Base



GOAL
Probe catalytic reactions on surfaces in real-time

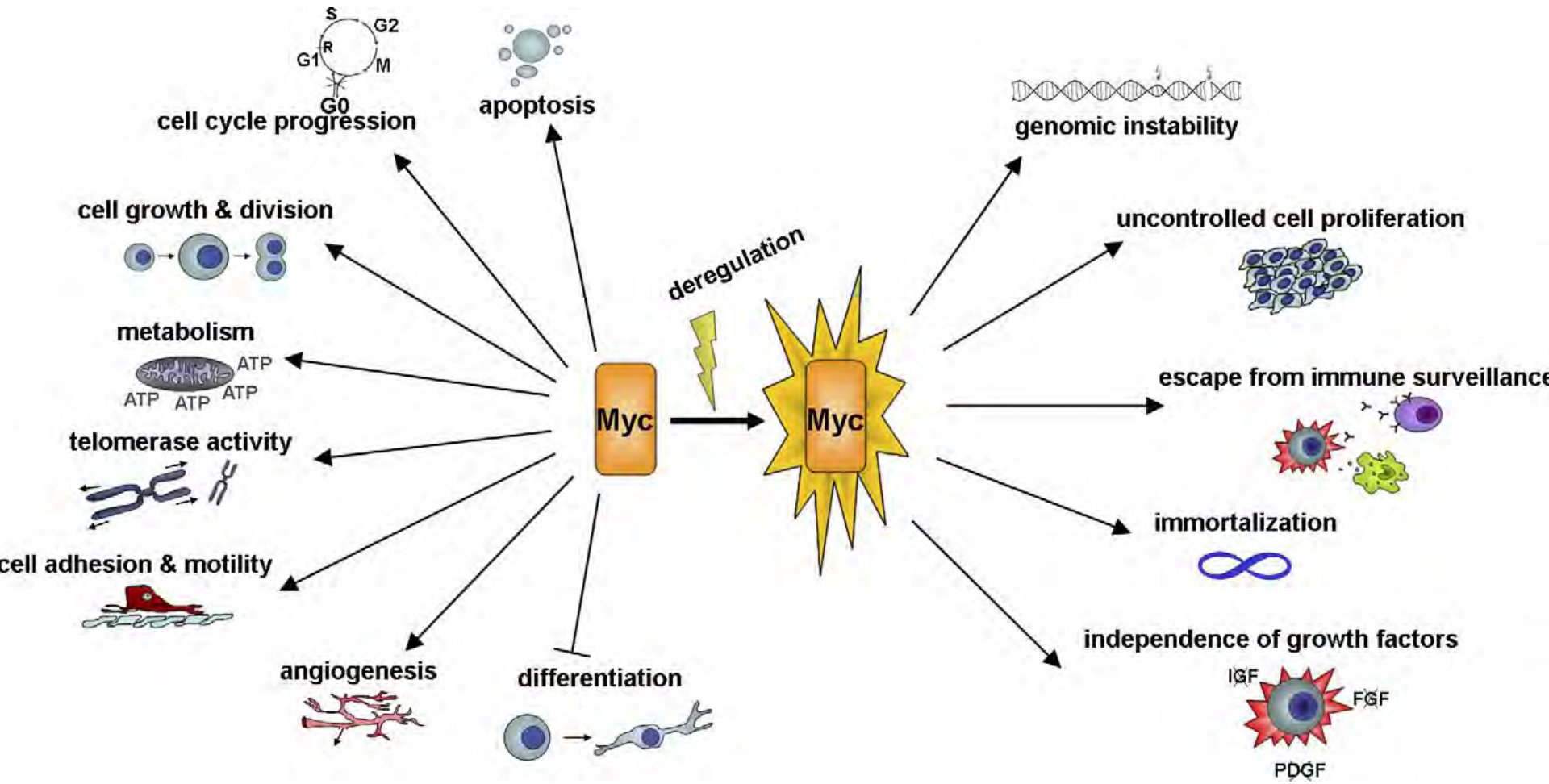
K. R. Brownell et al. manuscript in preparation.

Another application of DESI…

Biological Tissue Imaging MS

Deciphering the relationship between lipids and the *c*-MYC gene network…

The Many Faces of *c*-MYC

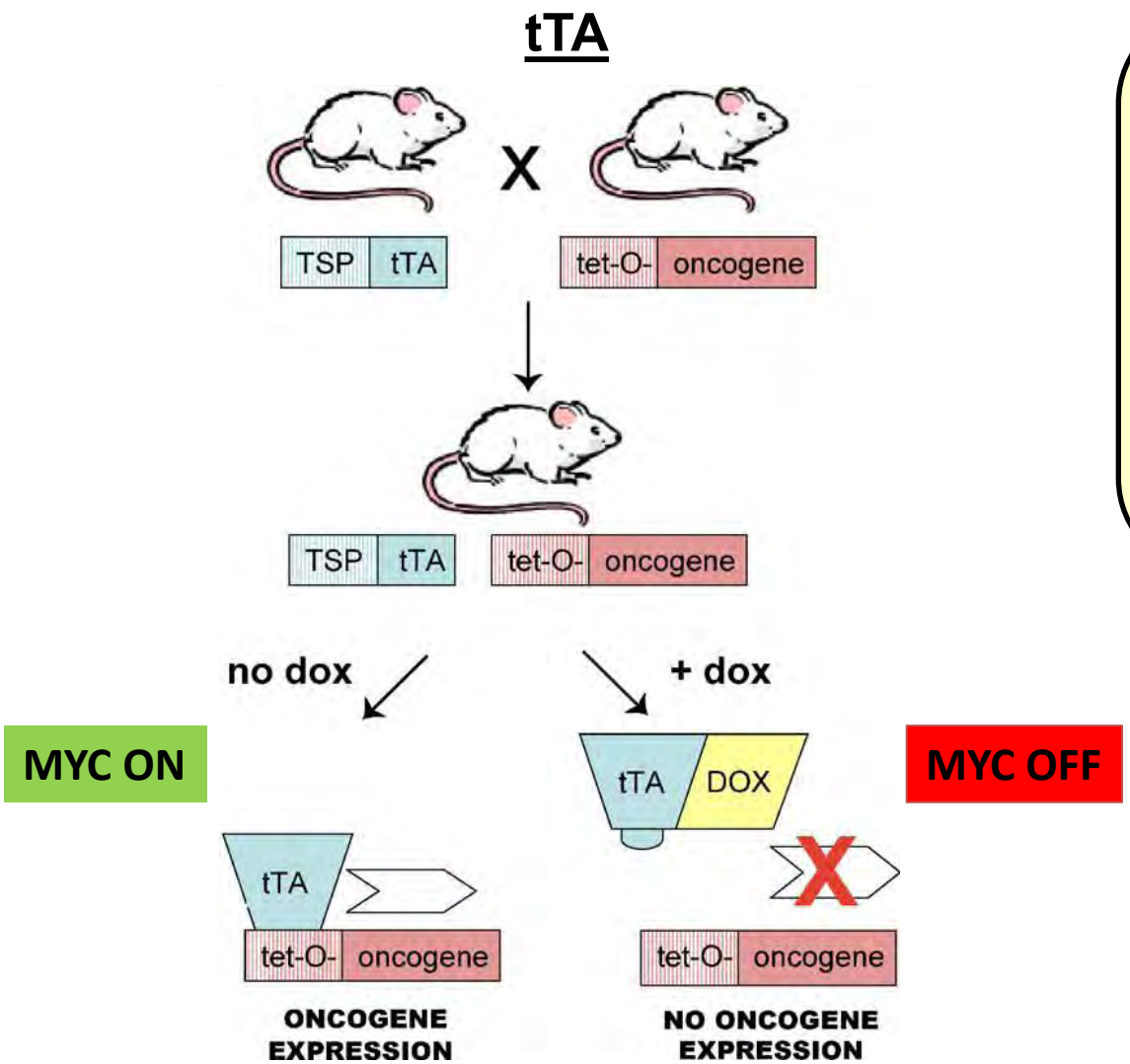


It is critical to understand the mode of action of MYC

Vita and Henriksson, *Semin. Cancer Biol.* **2006**, *16*, 318

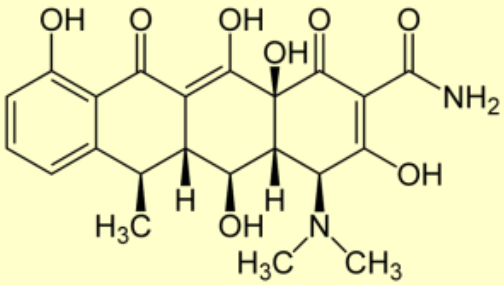
Studying *c*-MYC Expression in the Body

Conditional Transgenic Mouse Models



Tetracycline System

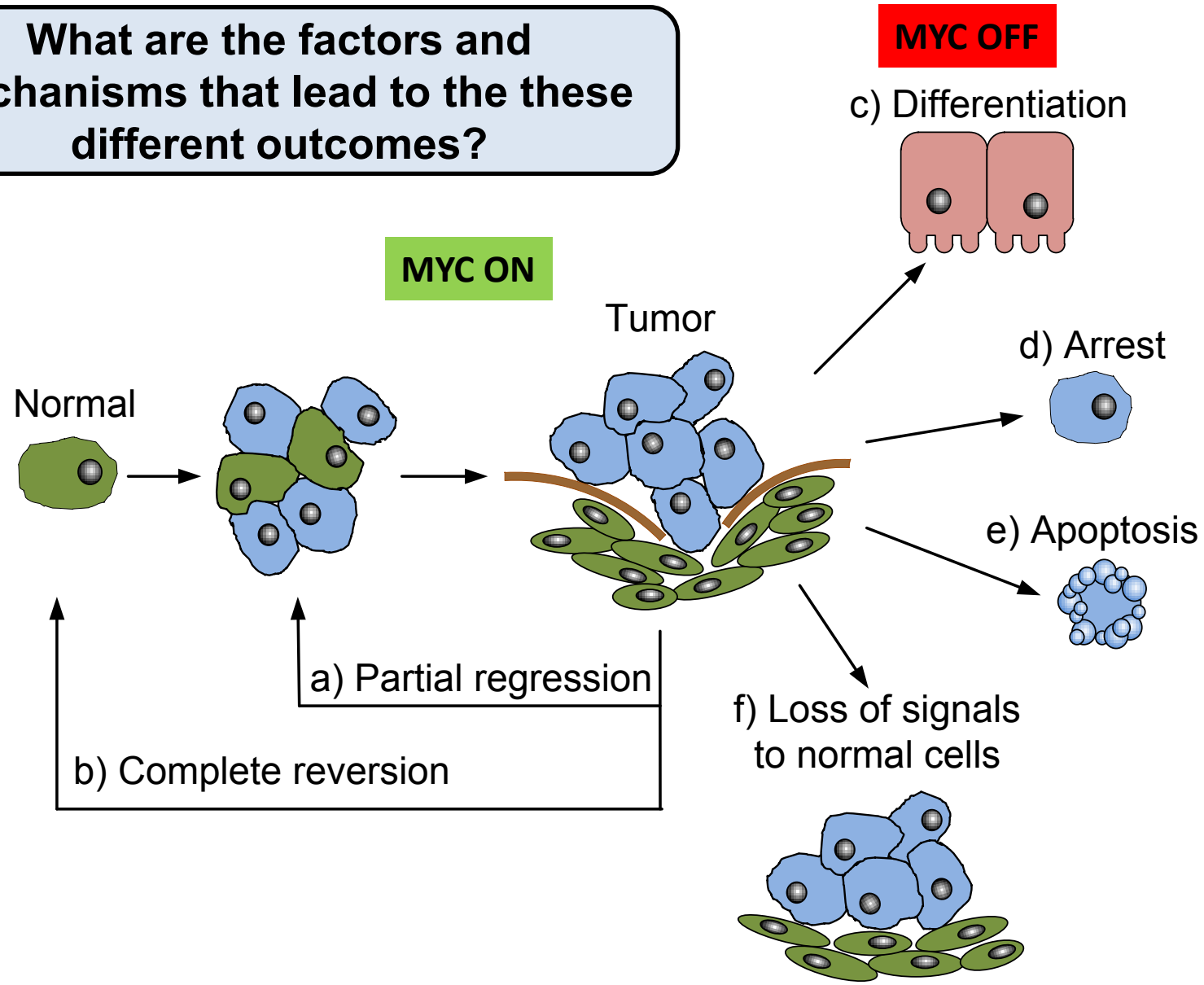
Doxycycline (dox)



Giuriato et al. *Semin. Cancer Biol.* **2004**, *14*, 3-11.

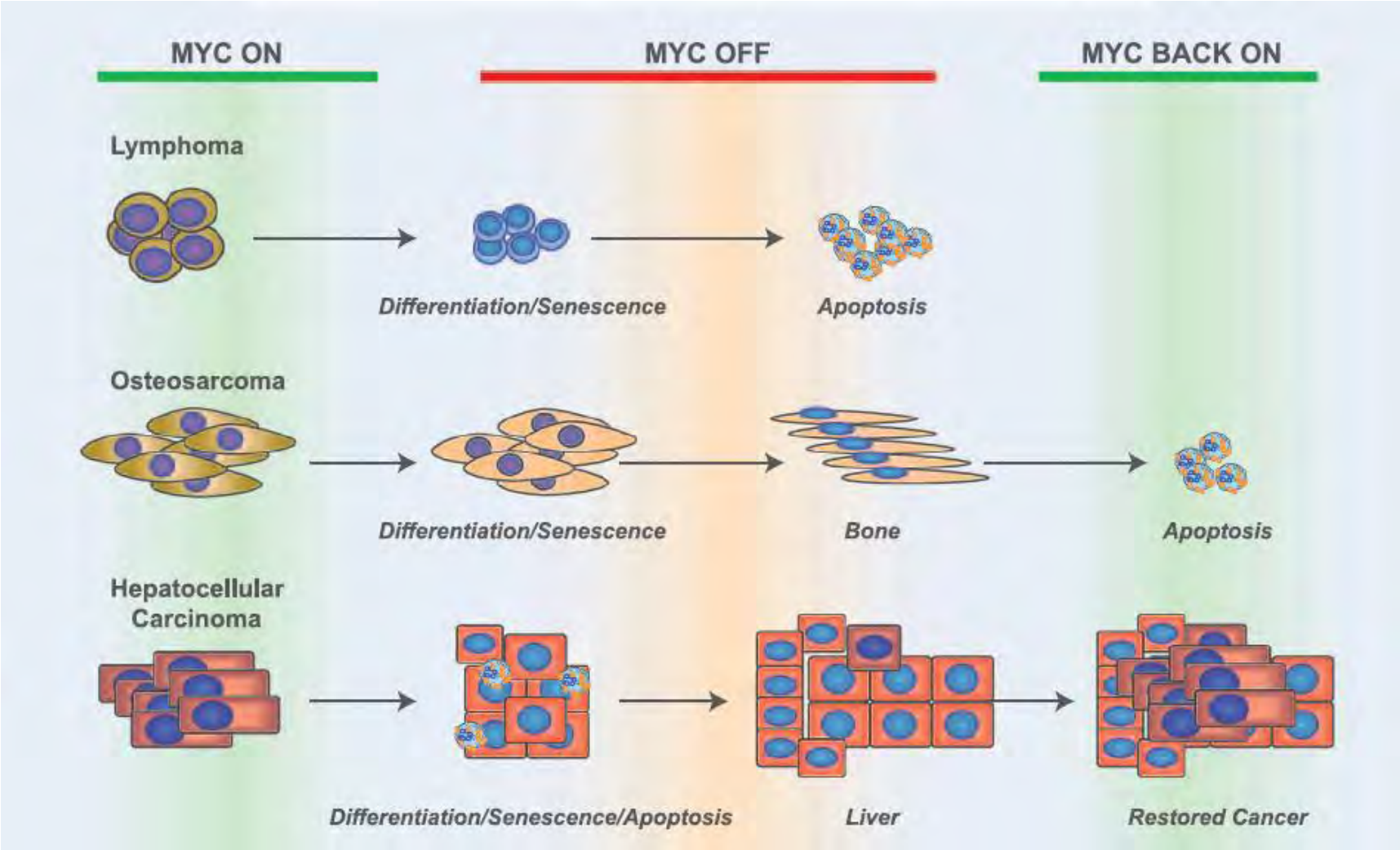
Outcomes of *c*-MYC Inactivation

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



Felsher, D. W.Cancer Res. **2008,** 68, 3081

Outcomes Depend on Tissue Type

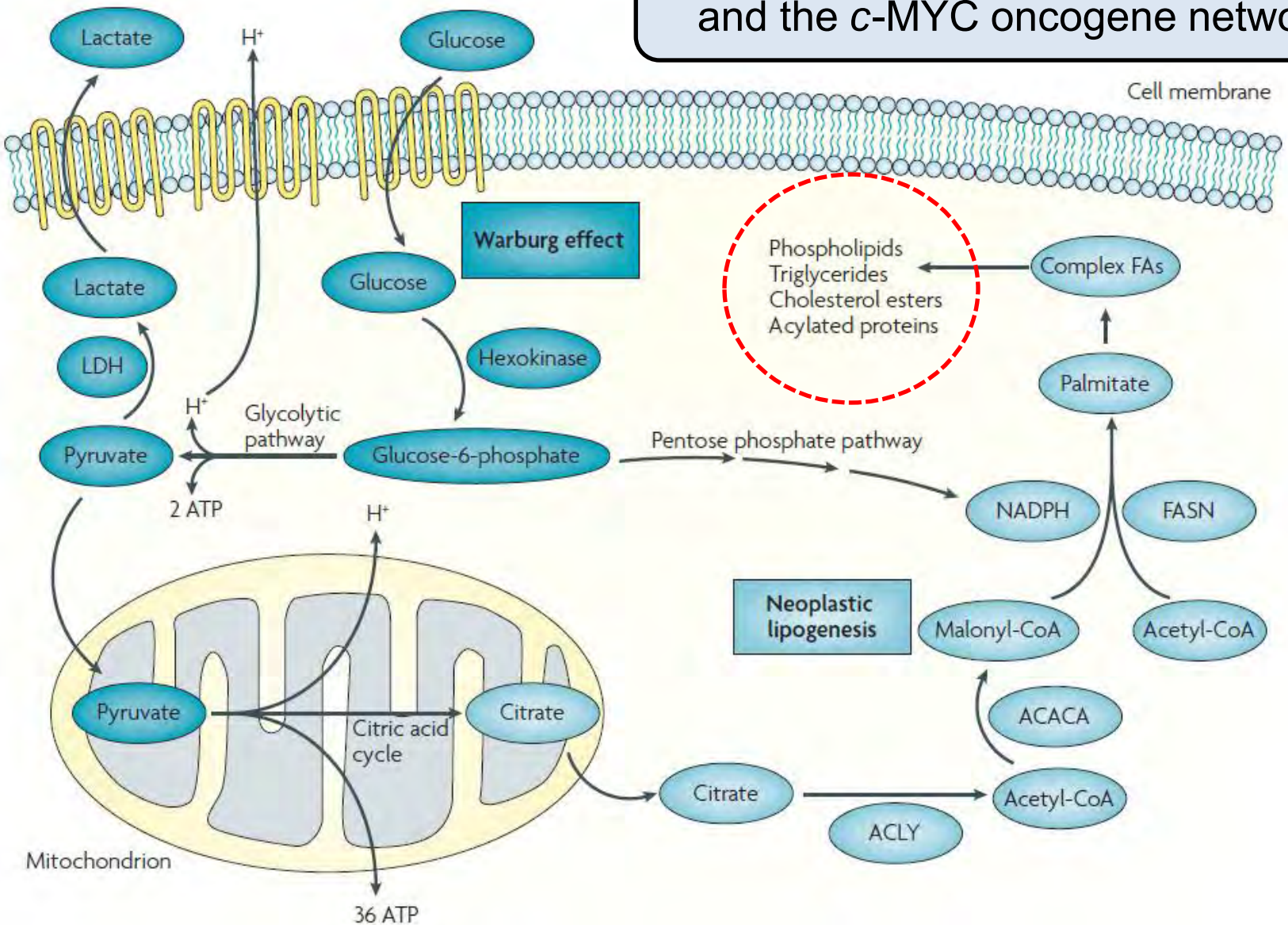


How does the tissue location affect the mechanism of *c*-MYC-induced cancer progression and regression?

Felsher, *Cancer Res.* **2008,** 68, 3081

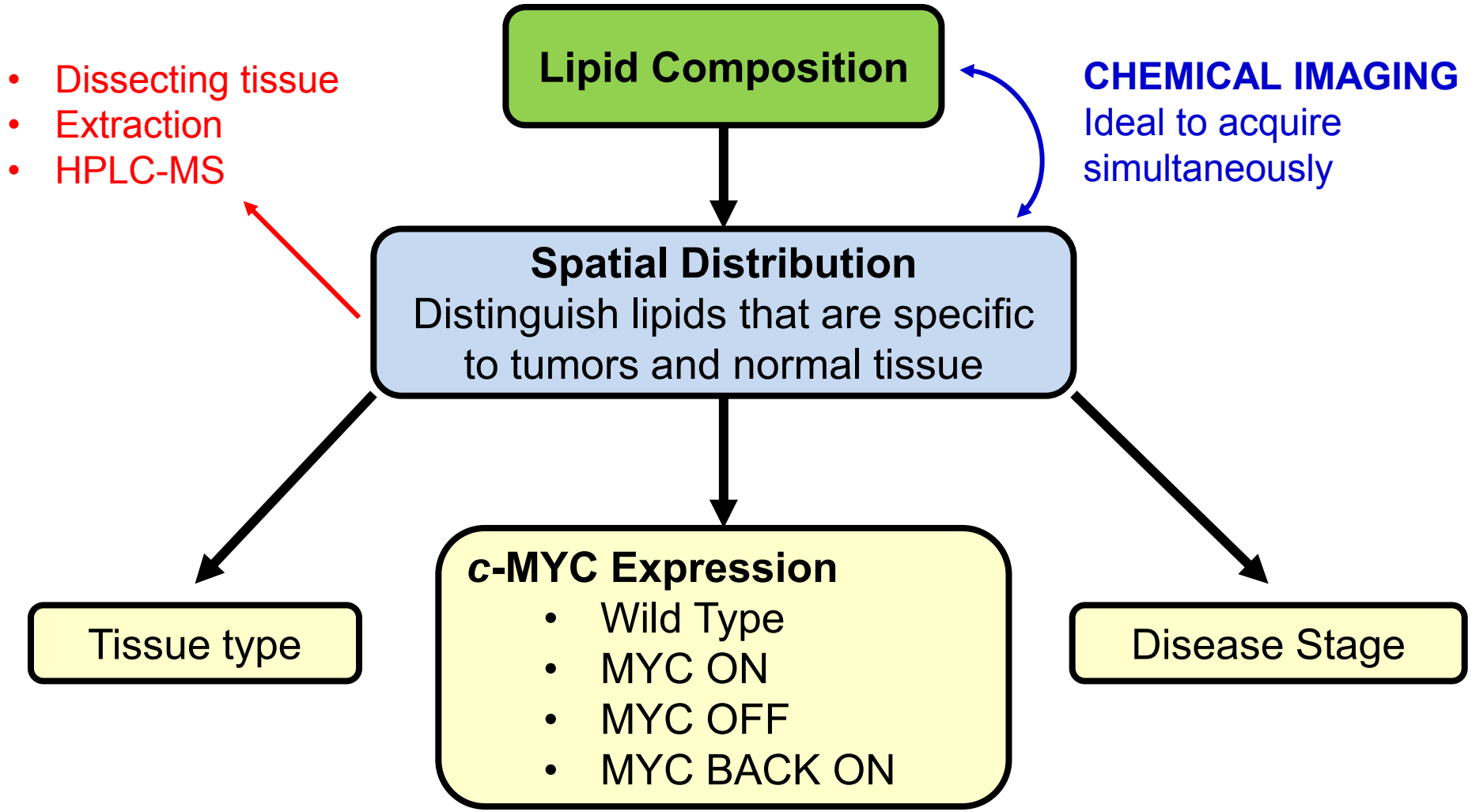
Lipids, the Warburg Effect, and Cancer

What is the relationship between lipids and the *c*-MYC oncogene network?

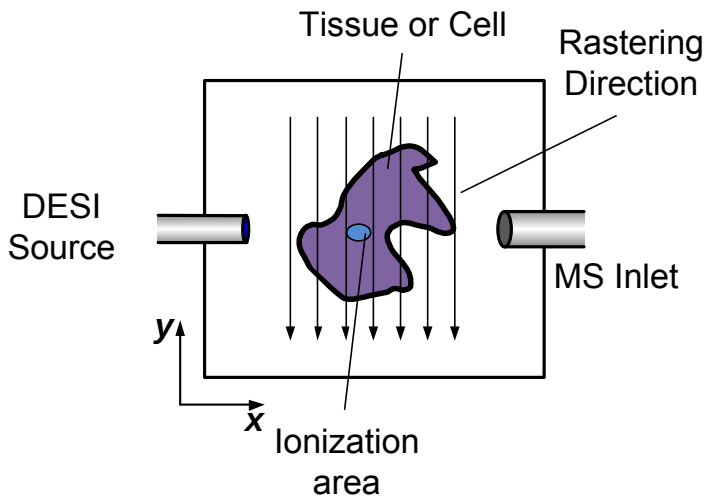


Menendez and Lupu, *Nat. Rev. Cancer* **2007**, *7*, 763

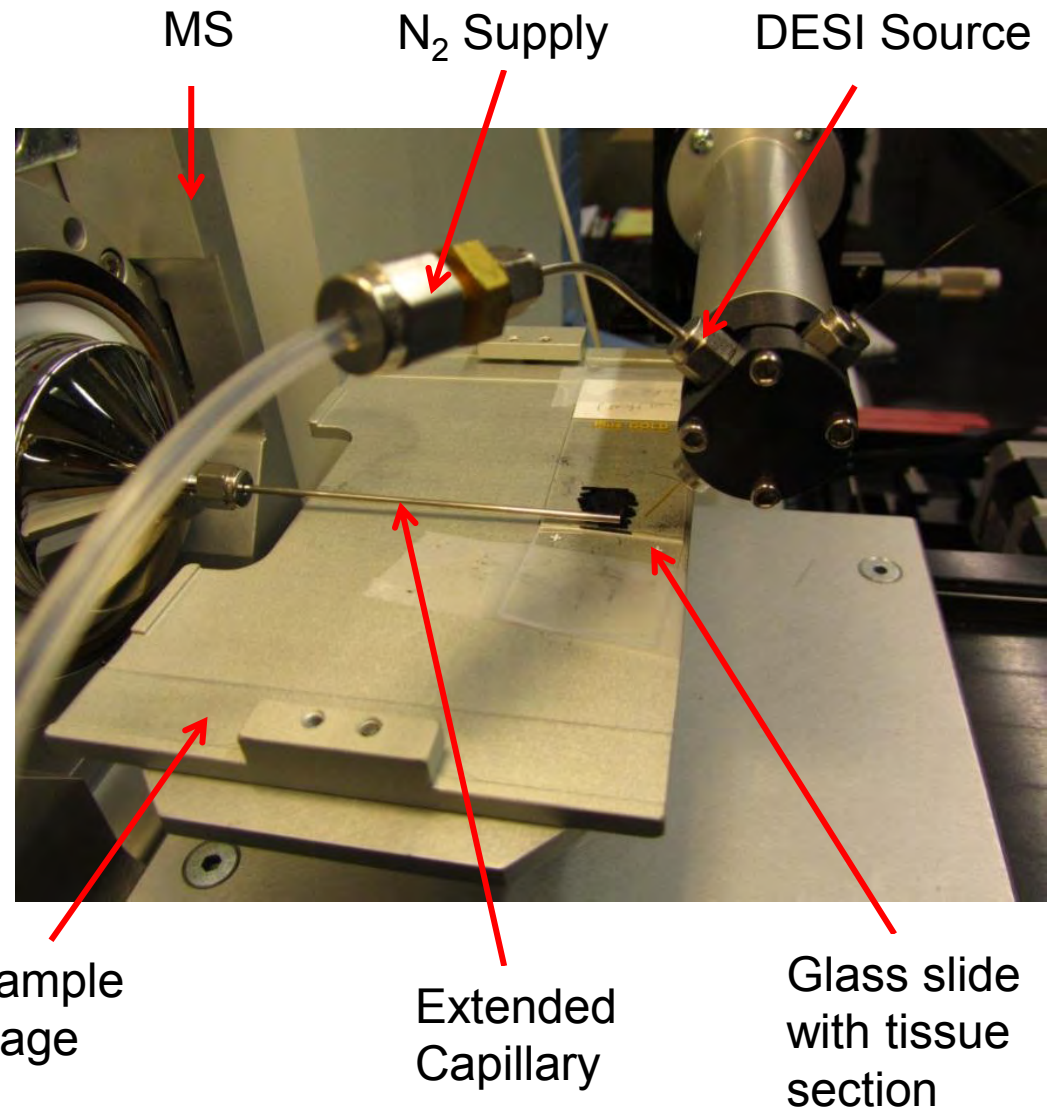
A simple approach to the problem…maybe…



DESI Imaging



Previous studies show that DESI imaging is great for detecting lipids in tissues



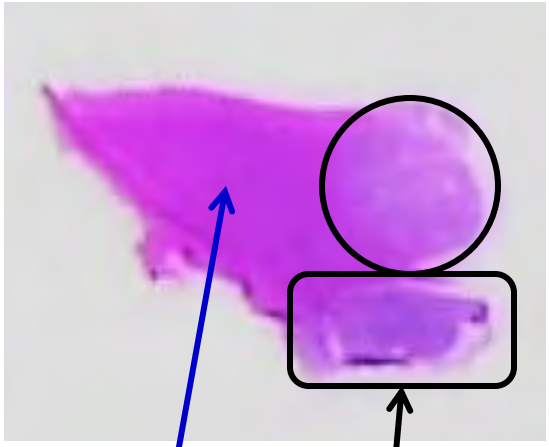
Ifa et al. *Int. J. Mass Spectrom.* **2007**, *259*, 8

Eberlin et al. *Angew. Chem.-Int. Edit.* **2010**, *49*, 873

What type of information can we obtain using DESI imaging MS?

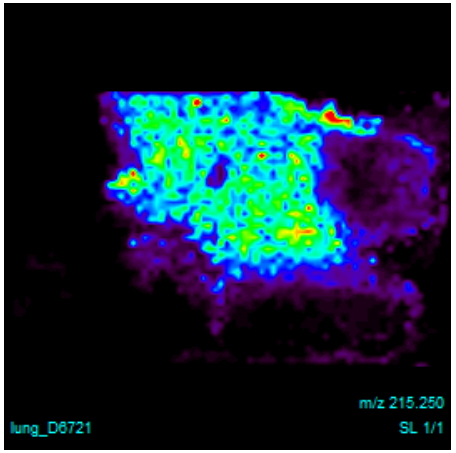
Lipid Species Only Present in Normal Tissue

Liver: *c*-MYC Activated for 4 Months

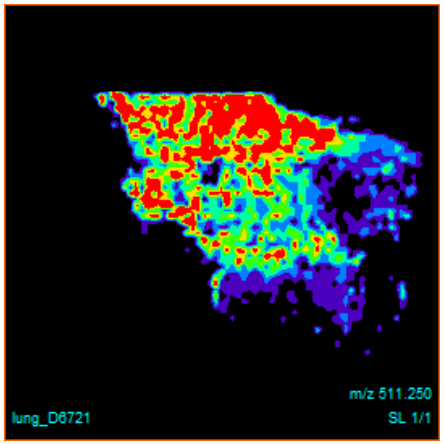


Tumors

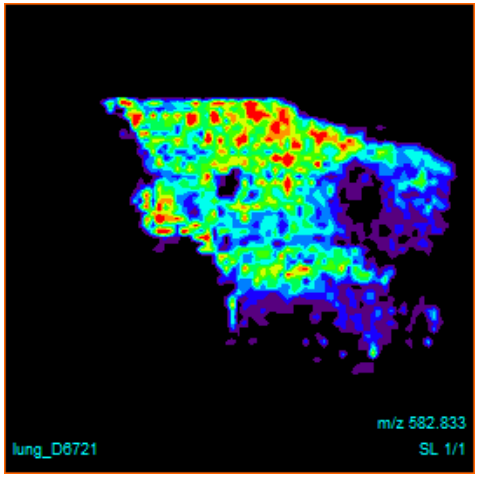
'Normal' (Adjacent)



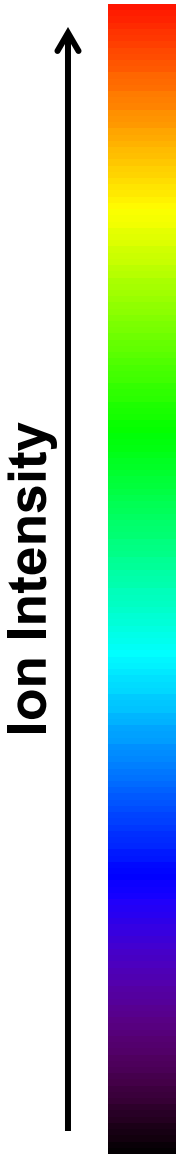
***m/z* 215**



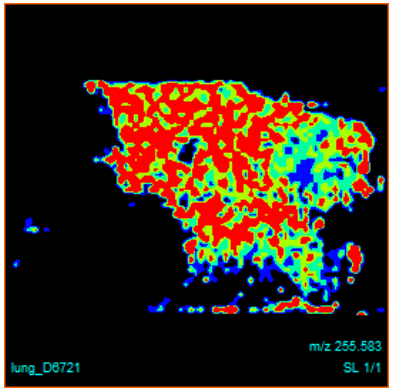
***m/z* 511**



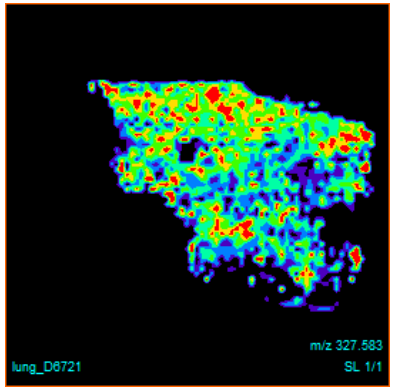
***m/z* 583**



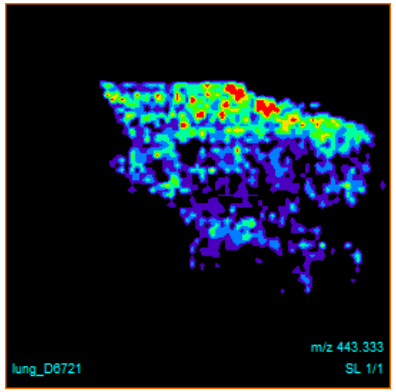
Lipid Species Present in Normal and Cancer Tissue



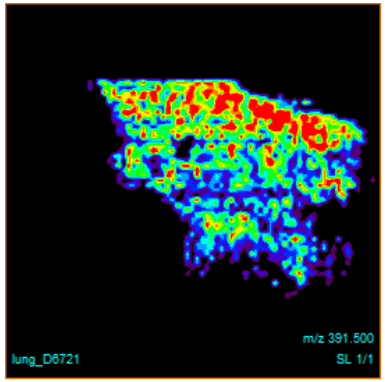
m/z 256



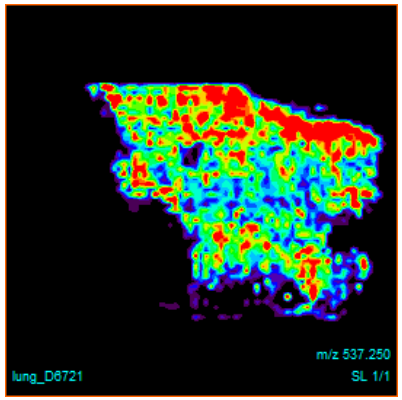
m/z 328



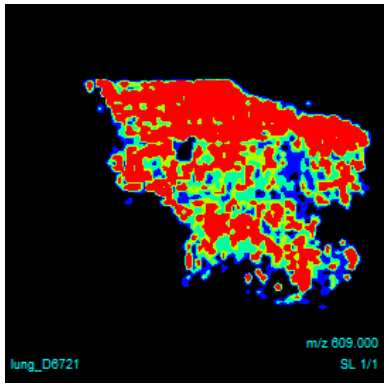
m/z 443



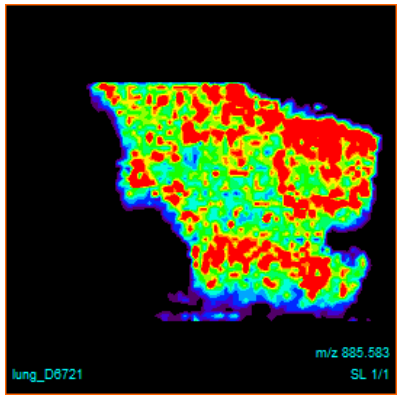
m/z 392



m/z 537



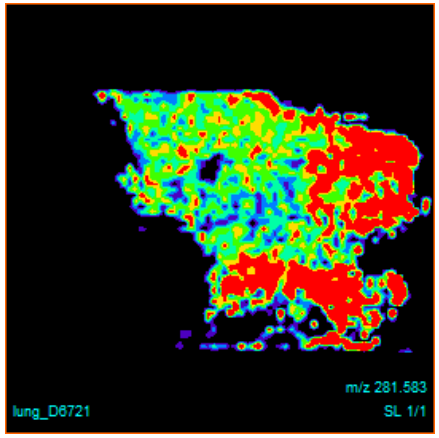
m/z 609



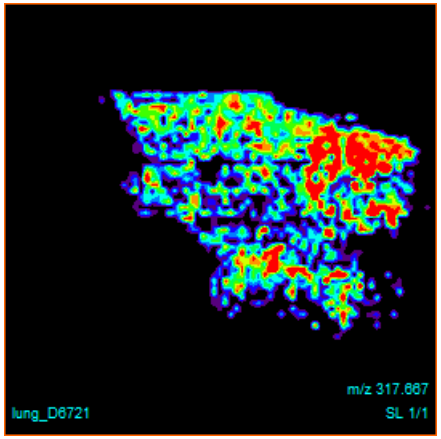
m/z 886

- **Images show that each species has a different intensity profile across the tissue**

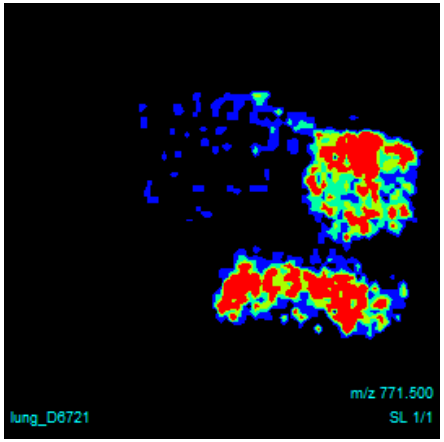
Lipid Species Present in Both Tumor Regions



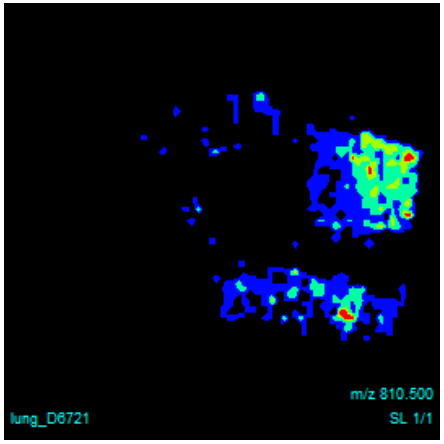
***m/z* 282**



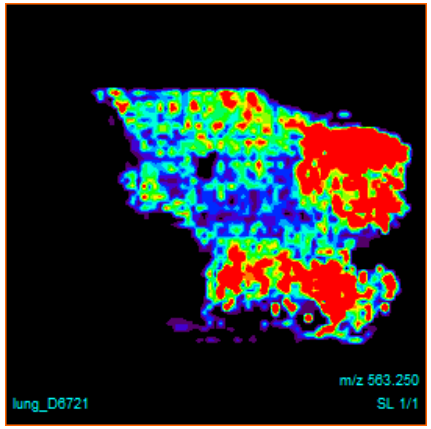
***m/z* 318**



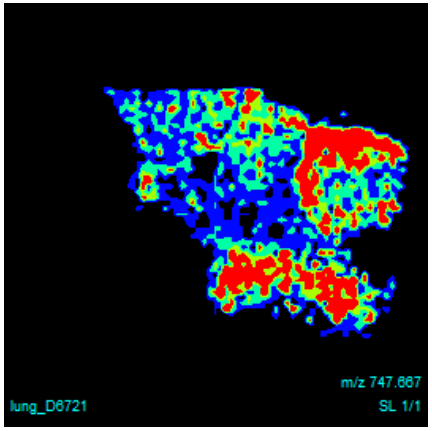
***m/z* 772**



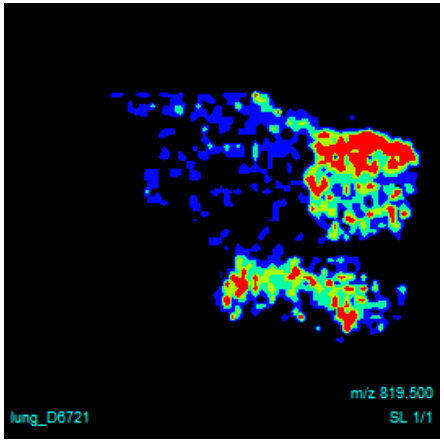
***m/z* 810**



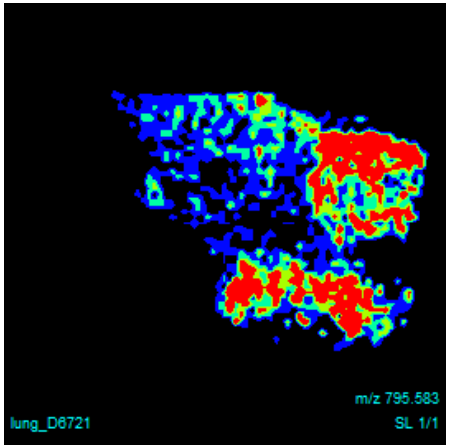
***m/z* 563**



***m/z* 748**

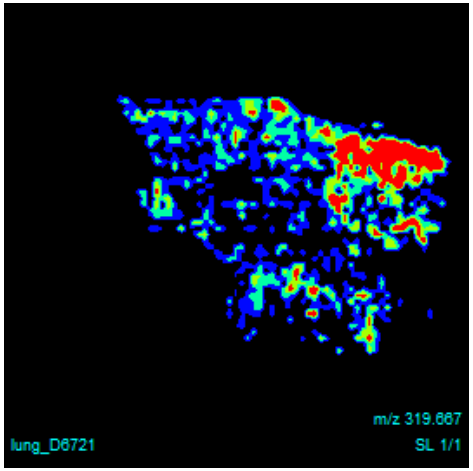


***m/z* 820**

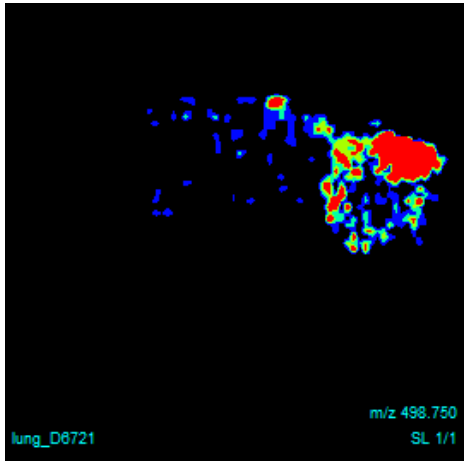


***m/z* 796**

Lipid Species Present in Only One Tumor Tissue

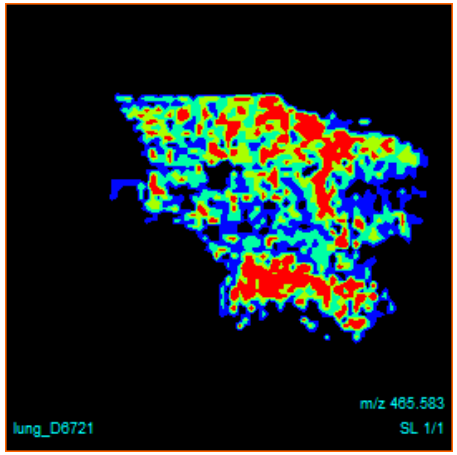


m/z 320

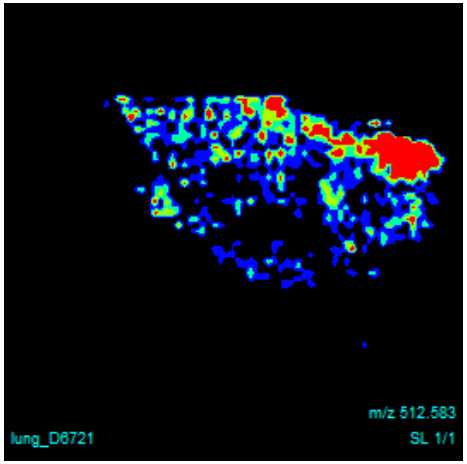


m/z 499

Different genotypes?

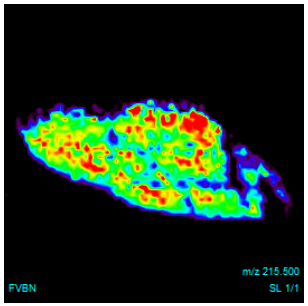


m/z 466

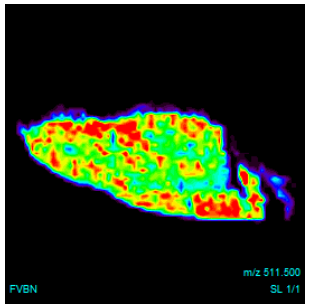


m/z 513

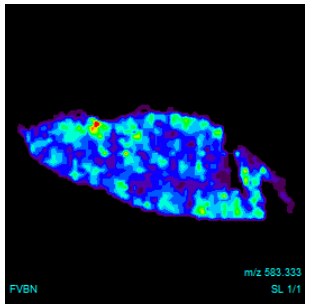
Lipid Species in Wild Type Tissue



m/z 215

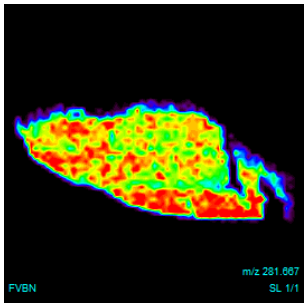


m/z 511

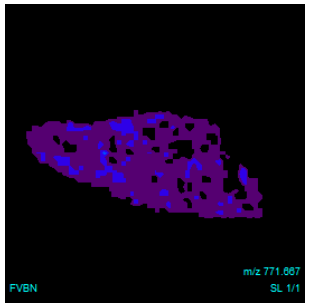


m/z 583

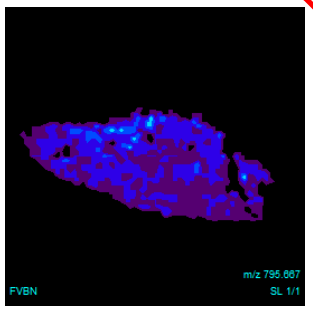
Down-regulated in tumors



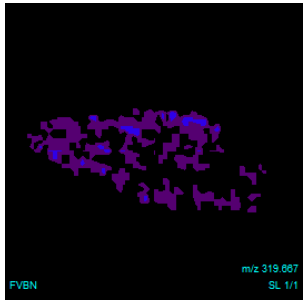
m/z 282

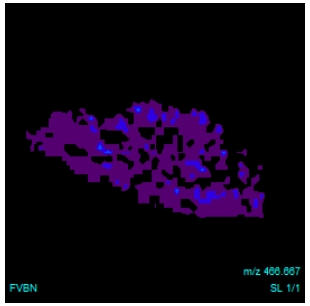


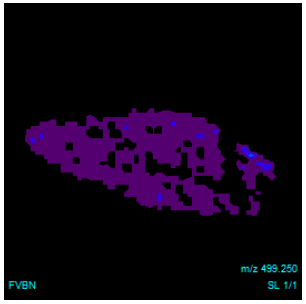
m/z 772



m/z 796

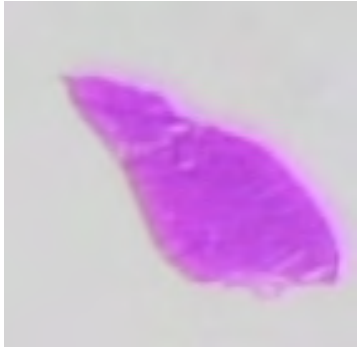




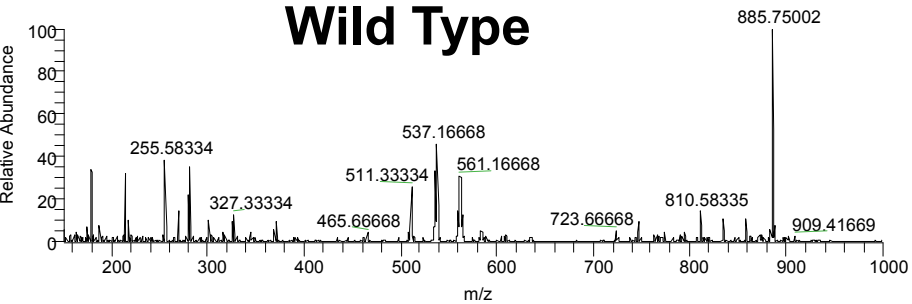


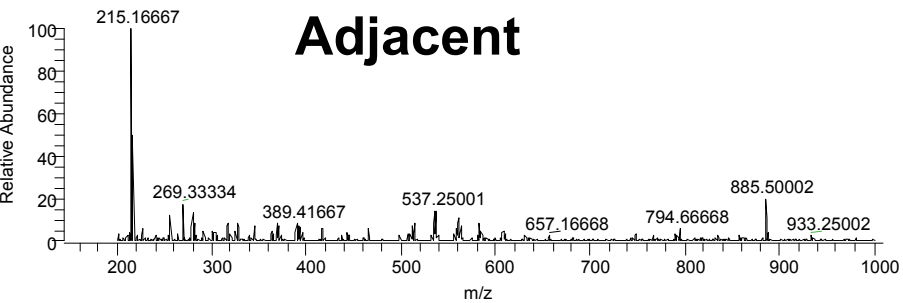
Up-regulated in tumors

Optical Image

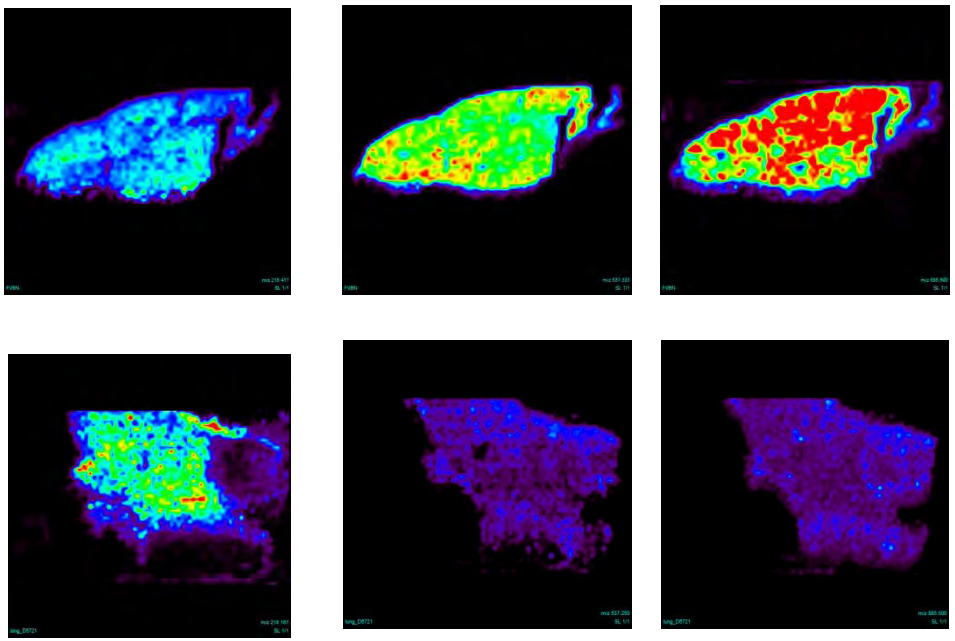


Comparison of Wild Type and Adjacent Tissues





Plotted on the same intensity scale

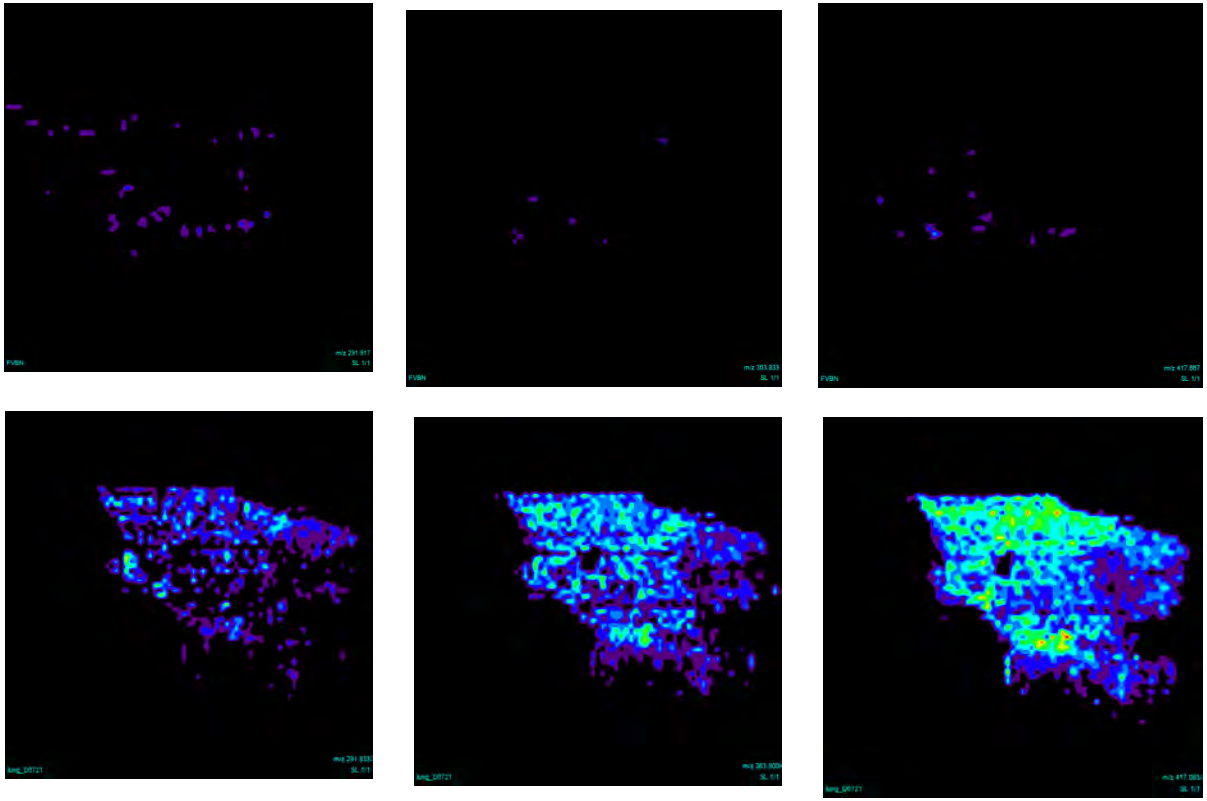


***m/z* 215** ***m/z* 537** ***m/z* 886**

Plotted on the same intensity scale

Wild Type

Adjacent

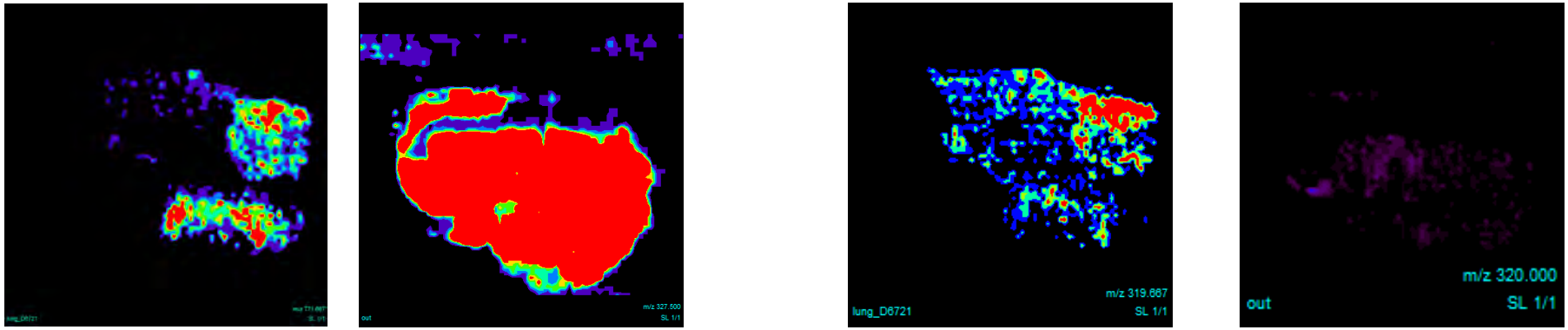


***m/z* 292** ***m/z* 364** ***m/z* 417**

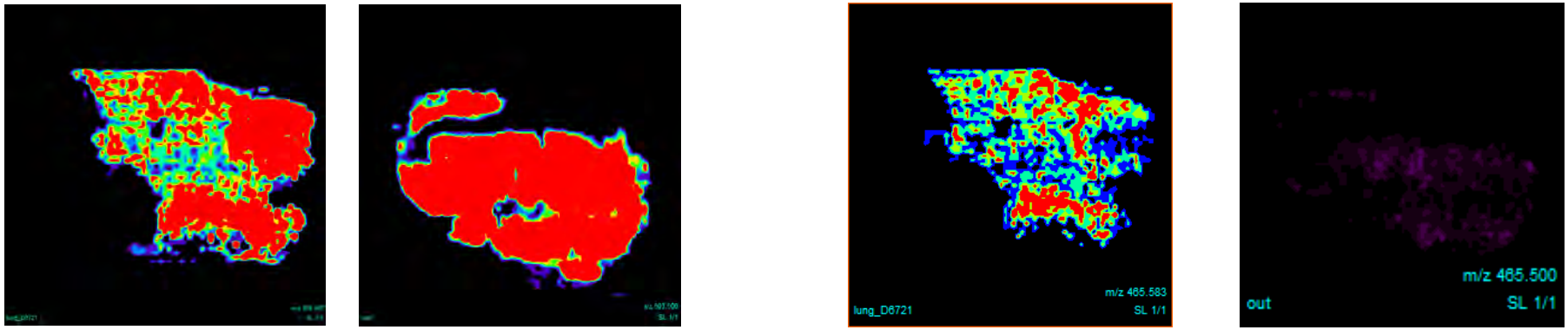
- **Cells in the adjacent tissue are affected by the tumors – i.e. not normal**
- **Species tumors release into the extracellular space?**

Late Stage Tumor

Early **Late** **Early** **Late**



***m/z* 772** ***m/z* 320**

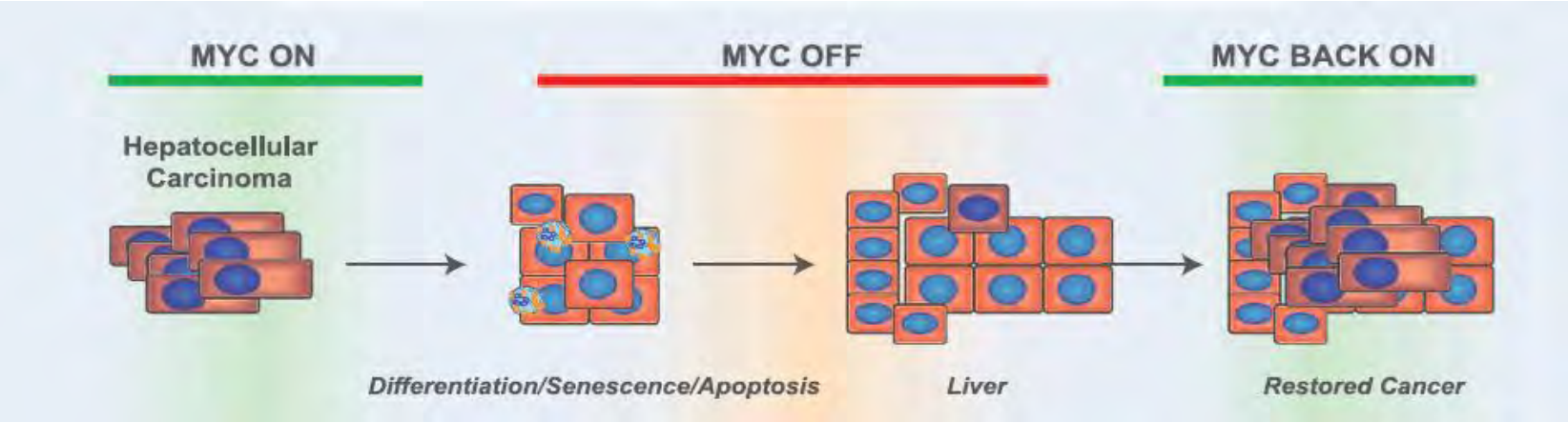


***m/z* 563** ***m/z* 466**

Observe time course of each lipid

Identifying the detected species…

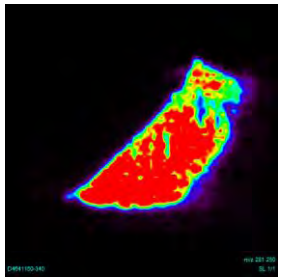
Just a reminder…



Fatty Acids

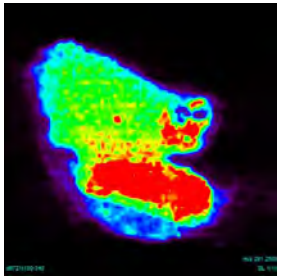
Wild Type

6 wks



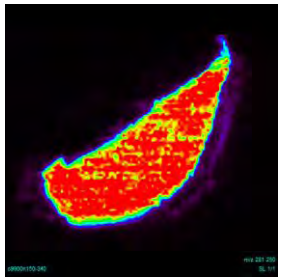
MYC ON

4 mos



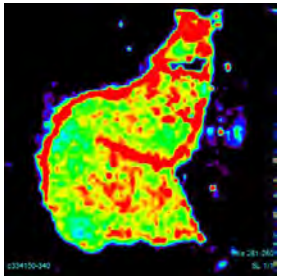
MYC OFF

6 mos

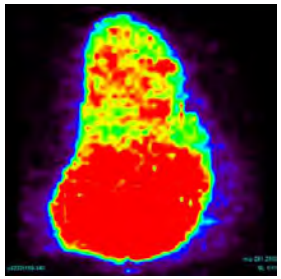


MYC ON

2 mos

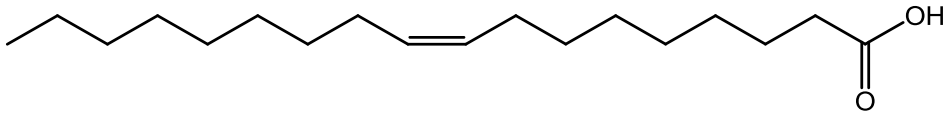


Late Stage Tumor



Difference between wild type and adjacent tissue – Warburg effect?

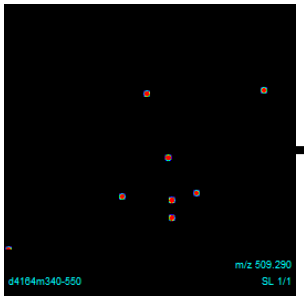
18:1



Monoacylglycerophosphoglycerols

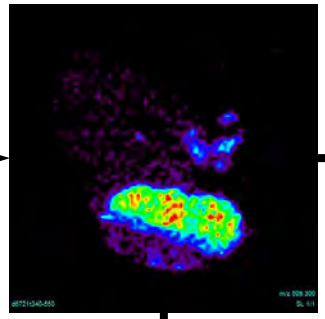
Wild Type

6 wks



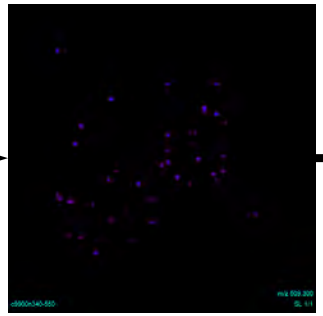
MYC ON

4 mos



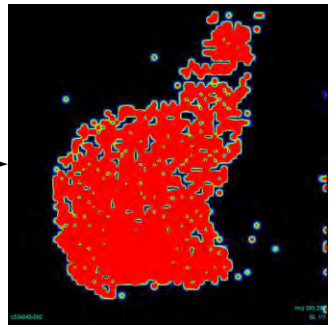
MYC OFF

6 mos

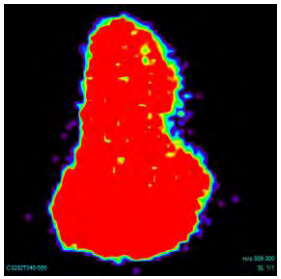


MYC ON

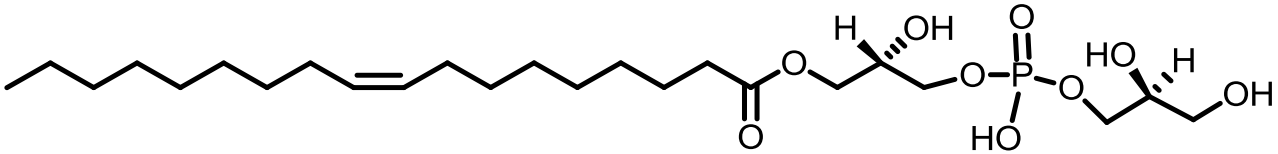
2 mos



Late Stage Tumor



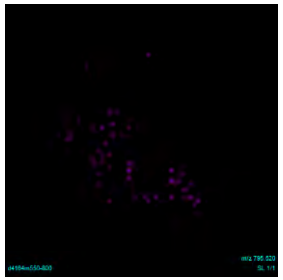
18:1
16:0
20:3



Diacylglycerophosphoglycerols

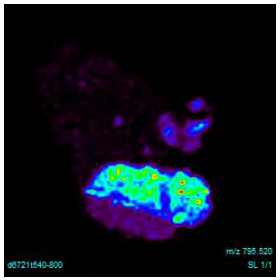
Wild Type

6 wks



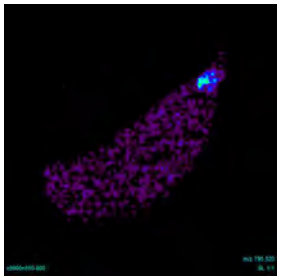
MYC ON

4 mos



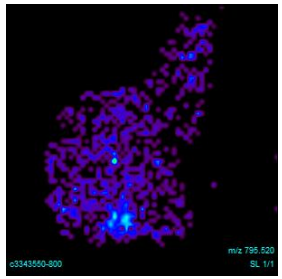
MYC OFF

6 mos

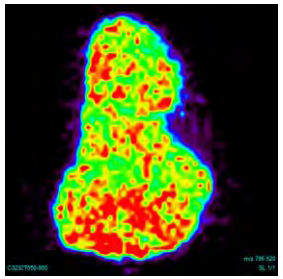


MYC ON

2 mos

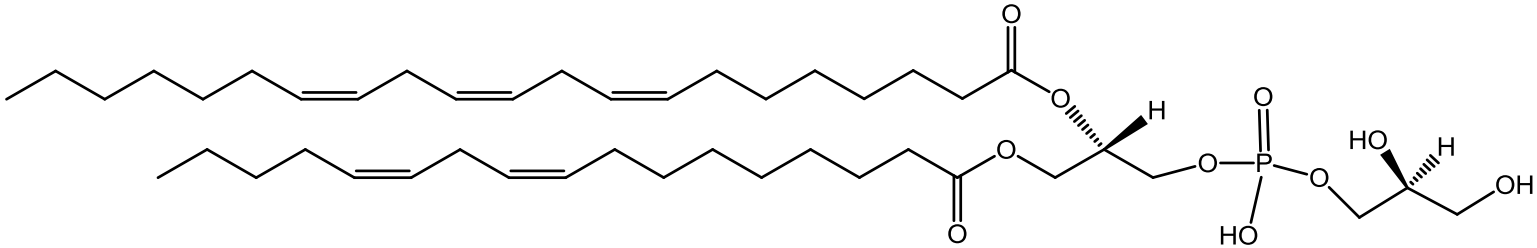


Late Stage Tumor



Different kinetics!

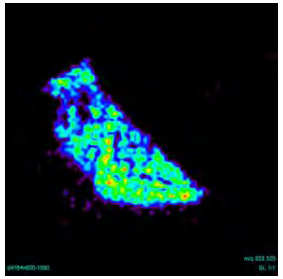
18:2/20:3
18:1/20:2
20:3/20:4
20:4/22:6



Diacylglycerophosphoinositols

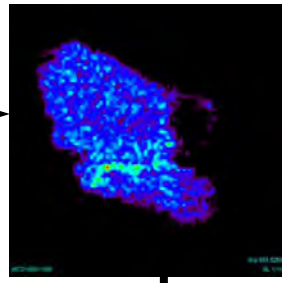
Wild Type

6 wks



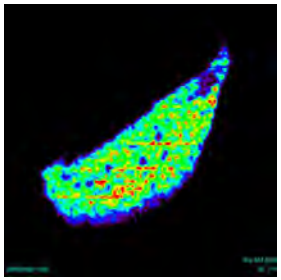
MYC ON

4 mos



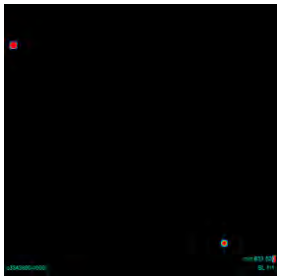
MYC OFF

6 mos

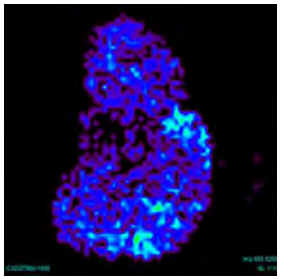


MYC ON

2 mos

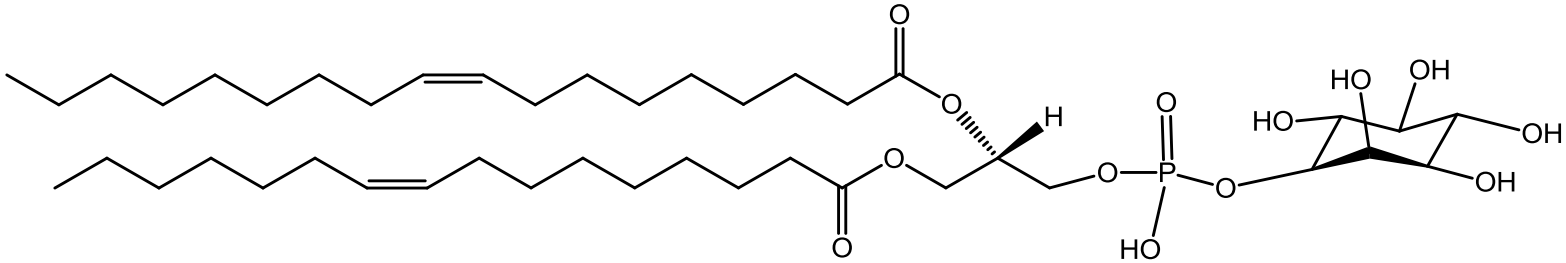


Late Stage Tumor



Down-regulation in the entire tissue

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

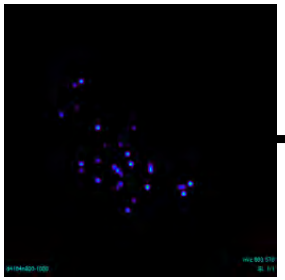


18:1/18:1 Diacylglycerophosphoinositol

Early stage lipid species!

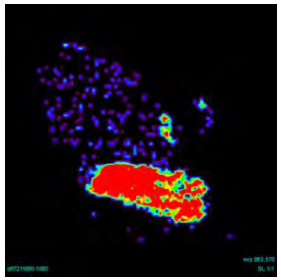
Wild Type

6 wks



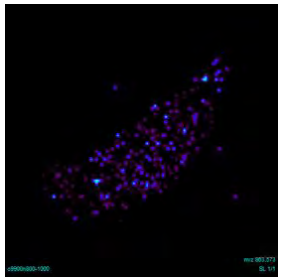
MYC ON

4 mos



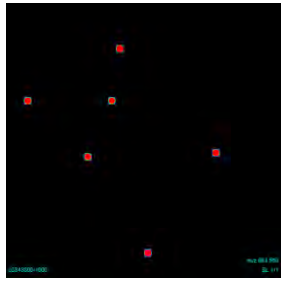
MYC OFF

6 mos

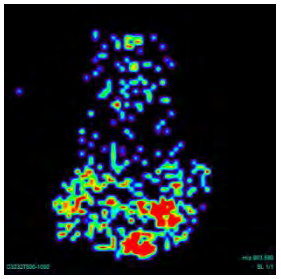


MYC ON

2 mos



Late Stage Tumor



The people who make anything possible…

Prof. Richard N. Zare
Dr. Konstantin Chingin
Dr. Ali Ismail
Thomas J. Cahill, III

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

C-H Amination
Prof. Justin Du Bois
Jennifer Roizen

MS Instrumentation
Dr. Allis Chien
Dr. Pavel Aronov

Ir WOC (Yale team)
Prof. Robert Crabtree
Prof. Mark Johnson
Nathan Schley

Biological Imaging
Prof. Dean W. Felsher
Dr. Emelyn Shroff
Dr. Tahera Zabuawala
Dr. David Bellovin

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