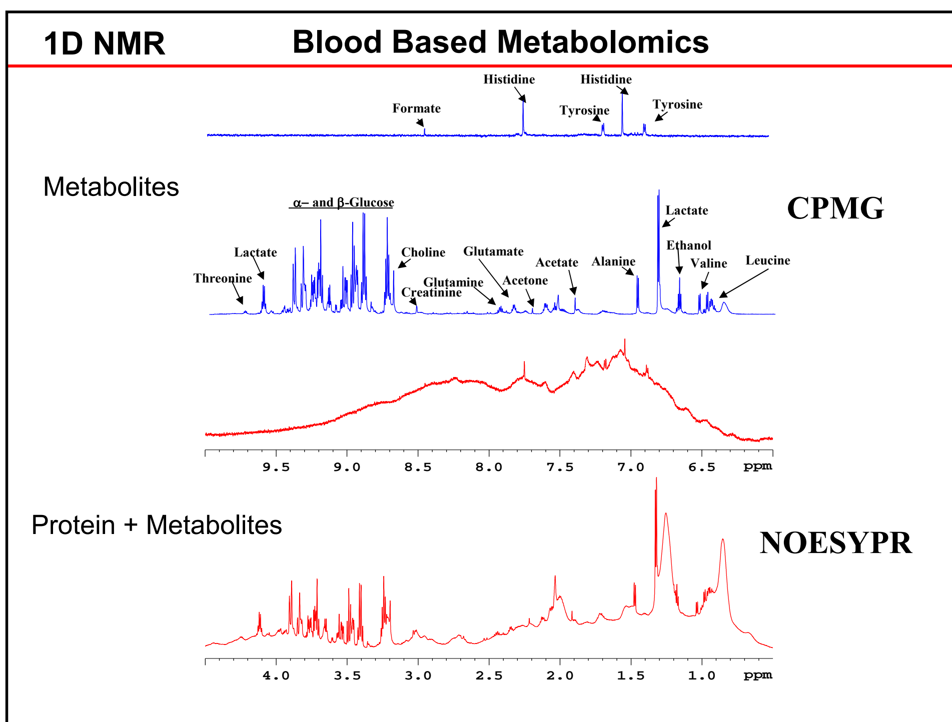
Nuclear Magnetic Resonance (NMR) Spectroscopy

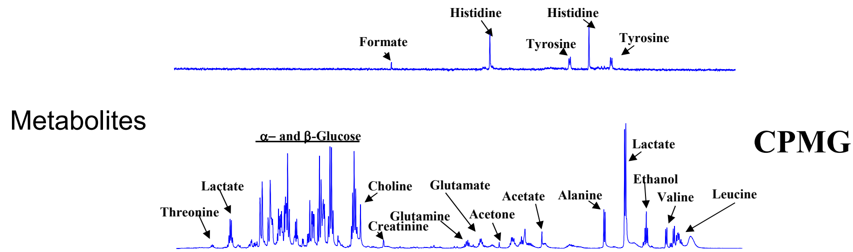
G. A. Nagana Gowda
Northwest Metabolomics Research Center
University of Washington, Seattle, WA

1D NMR Blood Based Metabolomics



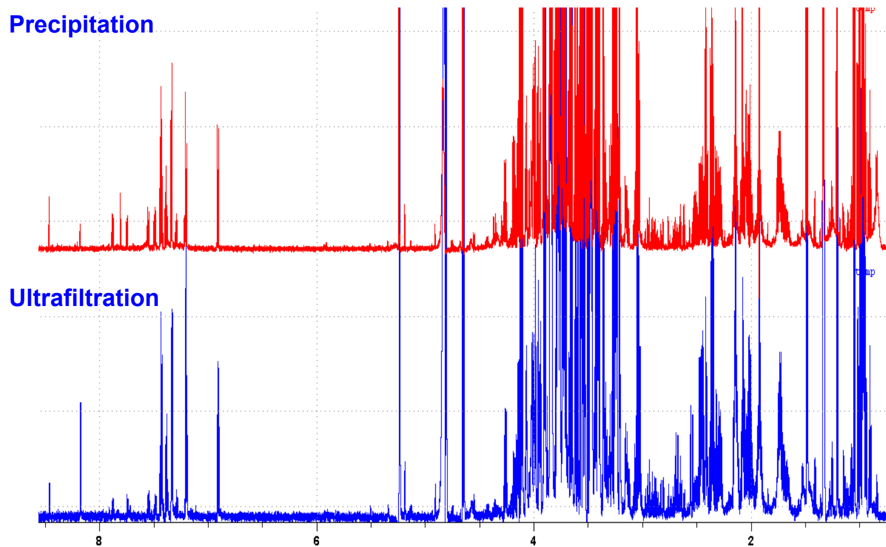
Limitations of Intact Blood serum/plasma Analysis

Metabolites

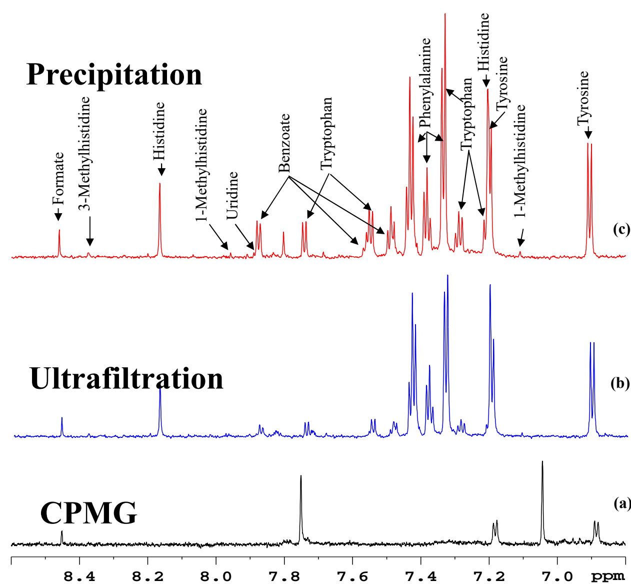


- Far fewer number of metabolites ~ 30 or less
- Suppression or attenuation of metabolite peaks due to protein binding

Alternative Blood Analysis Methods



Comparison of Blood Metabolite Analysis Methods



Anal Chem. 2014 Jun 3;86(11):5433-40

Suppression: Physically removing large molecules

<u>Metabolites Extraction using protein precipitation:</u>

Add methanol 2:1

Precipitate protein

Centrifuge, remove protein precipitate

Dry the supernatant and reconstitute in water

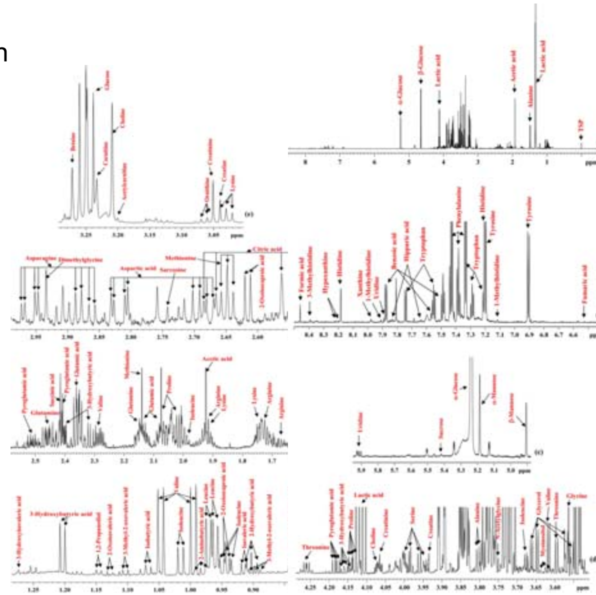
Filtration using molecular weight cut-off filters (3kDa)

Extracted samples can be used for both MS and NMR analyses

Anal Chem. 2015 Jan 6;87(1):706-15

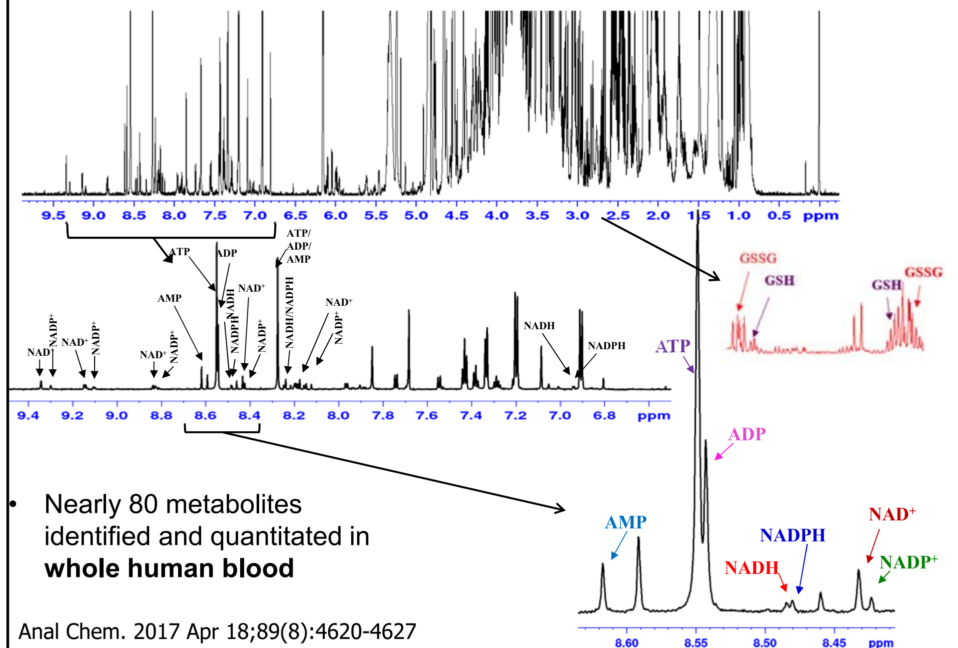
Identification and Quantitation of Blood Metabolites

- Nearly 70 metabolites identified and quantitated in **human blood plasma**



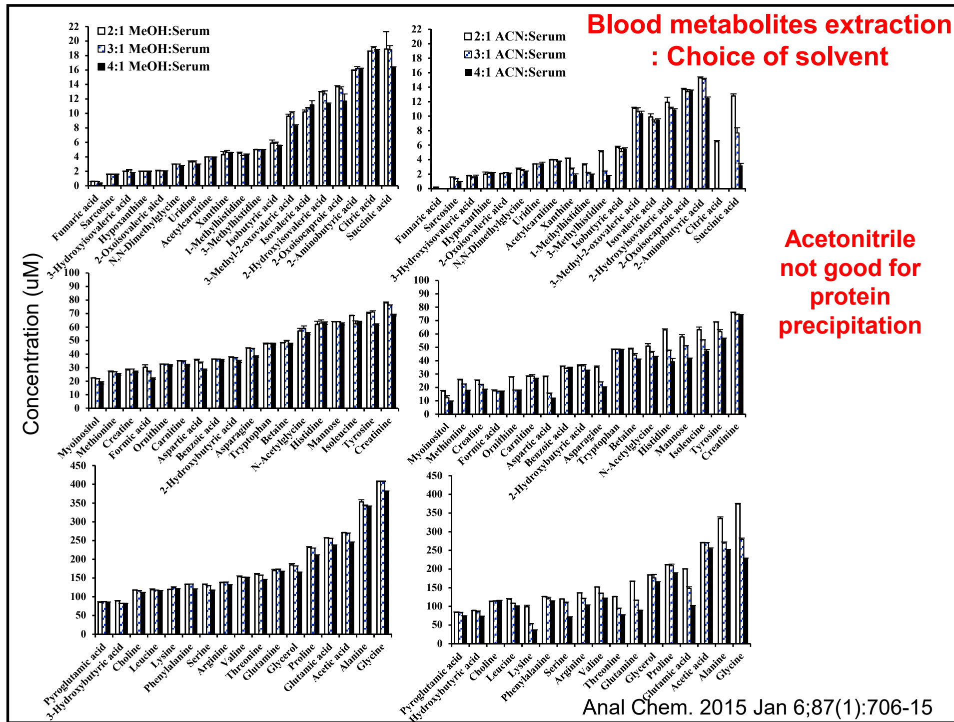
Anal Chem. 2015 Jan 6;87(1):706-15

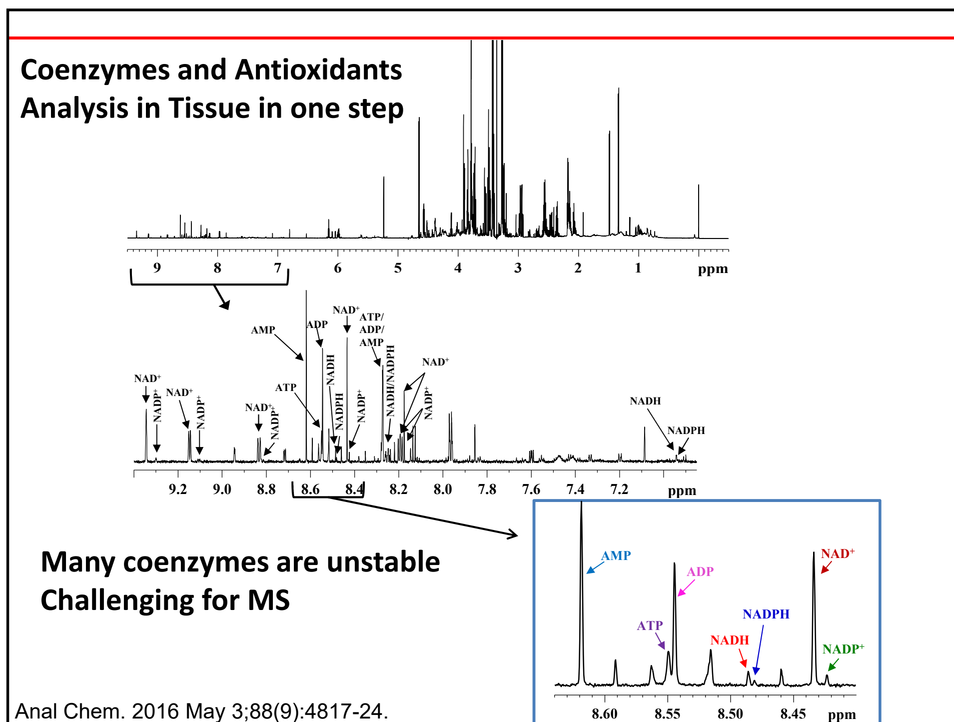
Whole Blood Metabolomics using NMR



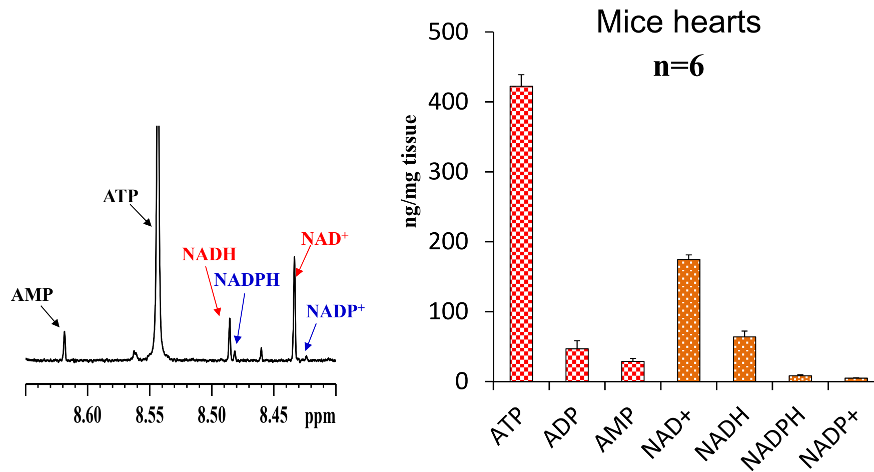
- Nearly 80 metabolites identified and quantitated in **whole human blood**

Anal Chem. 2017 Apr 18;89(8):4620-4627


Blood metabolites extraction
: Choice of solvent
Acetonitrile not good for protein precipitation
Concentration (uM)
2:1 MeOH:Serum
3:1 MeOH:Serum
4:1 MeOH:Serum
2:1 ACN:Serum
3:1 ACN:Serum
4:1 ACN:Serum
Anal Chem. 2015 Jan 6;87(1):706-15

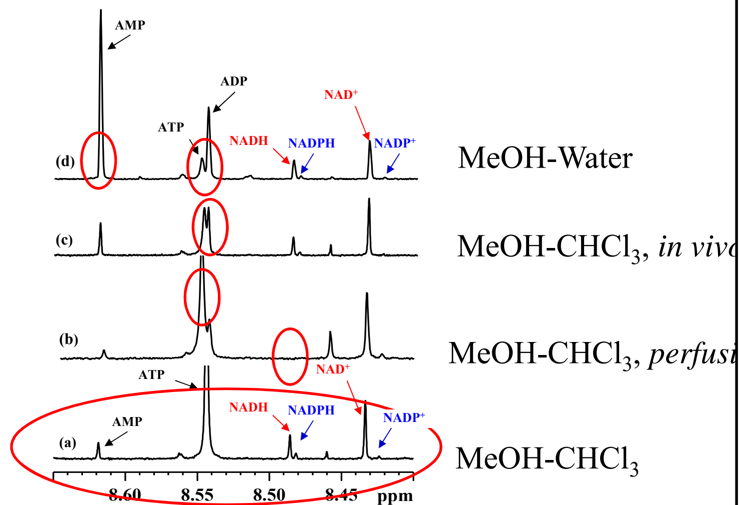

Coenzymes and Antioxidants Analysis in Tissue in one step
Many coenzymes are unstable
Challenging for MS
AMP
ADP
ATP
NADH
NADPH
NAD+
NADP+
ppm
Anal Chem. 2016 May 3;88(9):4817-24.

Simultaneous Analysis of Coenzymes



Sensitivity to Harvesting/Extraction

Mouse Heart



MeOH-Water

MeOH-$CHCl_3$, *in vivo*

MeOH-$CHCl_3$, *perfusi*

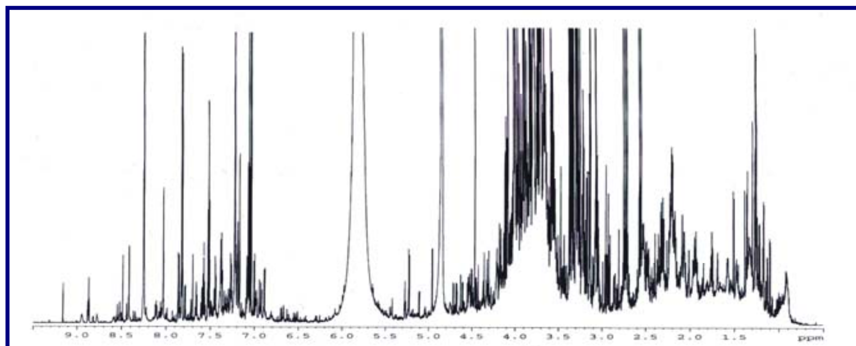
MeOH-$CHCl_3$

Urine NMR: Sample Preparation

Urine - Intact samples

No preparation required except adding buffer to stabilize pH

About 0.1% NaN_3 added to prevent bacterial growth



NATURE PROTOCOLS, 2(11), 2692-2703 (2007)

More number of metabolites detected by NMR than MS

OPEN ACCESS Freely available online PLOS | ONE

The Human Urine Metabolome

Souhaila Bouatra[1], Farid Aziat[1], Rupasri Mandal[1], An Chi Guo[2], Michael R. Wilson[2], Craig Knox[2], Trent C. Bjorndahl[1], Ramanarayan Krishnamurthy[1], Fozia Saleem[1], Philip Liu[1], Zerihun T. Dame[1], Jenna Poelzer[1], Jessica Huynh[1], Faizath S. Yallou[1], Nick Psychogios[3], Edison Dong[1], Ralf Bogumil[4], Cornelia Roehring[4], David S. Wishart[1,2,5]*

1 Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, 2 Department of Computing Sciences, University of Alberta, Edmonton, Alberta, Canada, 3 Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, 4 BIOCRATES Life Sciences AG, Innsbruck, Austria, 5 National Institute for Nanotechnology, Edmonton, Alberta, Canada

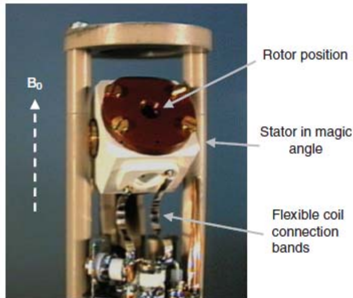
Abstract

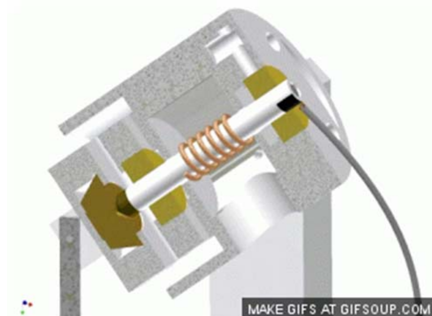
Urine has long been a "favored" biofluid among metabolomics researchers. It is sterile, easy-to-obtain in large volumes, largely free from interfering proteins or lipids and chemically complex. However, this chemical complexity has also made urine a particularly difficult substrate to fully understand. As a biological waste material, urine typically contains metabolic breakdown products from a wide range of foods, drinks, drugs, environmental contaminants, endogenous waste metabolites and bacterial by-products. Many of these compounds are poorly characterized and poorly understood. In an effort to improve our understanding of this biofluid we have undertaken a comprehensive, quantitative, metabolome-wide characterization of human urine. This involved both computer-aided literature mining and comprehensive, quantitative experimental assessment/validation. The experimental portion employed NMR spectroscopy, gas chromatography mass spectrometry (GC-MS), direct flow injection mass spectrometry (DFI/LC-MS/MS), inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC) experiments performed on multiple human urine samples. This multi-platform metabolomic analysis allowed us to identify 445 and quantify 378 unique urine metabolites or metabolite species. The different analytical platforms were able to identify (quantify) a total of: 209 (209) by NMR, 179 (85) by GC-MS, 127 (127) by DFI/LC-MS/MS, 40 (40) by ICP-MS and 10 (10) by HPLC. Our use of multiple metabolomics platforms and technologies allowed us to identify several previously unknown urine metabolites and to substantially enhance the level of metabolome coverage. It also allowed us to critically assess the relative strengths and weaknesses of different platforms or technologies. The literature review led to the identification and annotation of another 2206 urinary compounds and was used to help guide the subsequent experimental studies. An online database containing the complete set of 2651 confirmed human urine metabolite species, their structures (3079 in total), concentrations, related literature references and links to their known disease associations are freely available at http://www.urinemetabolome.ca.

September 2013 | Volume 8 | Issue 9 | e73076

Preparation of Cells and Tissue for NMR

Magic angle spinning for intact cells and tissue samples

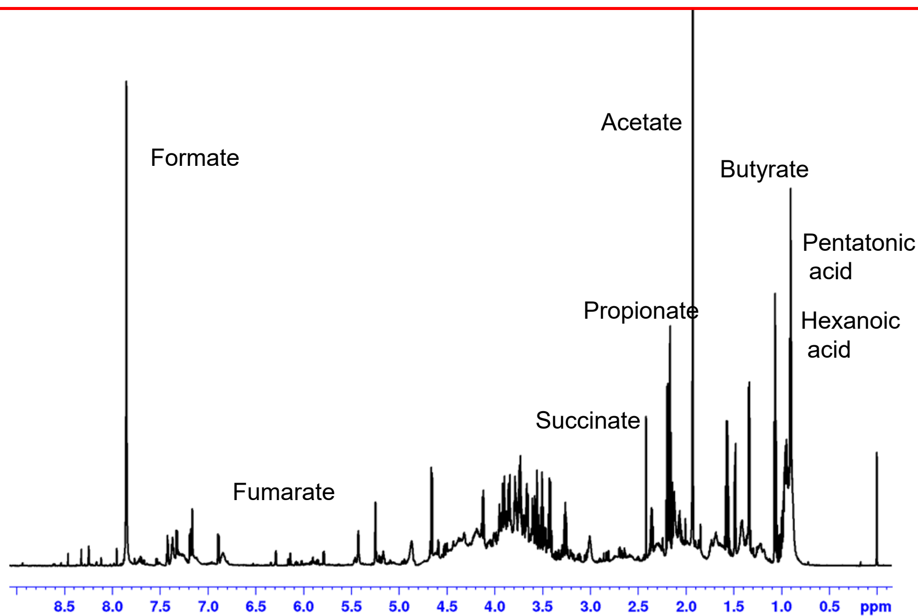




54.7° Magic Angle

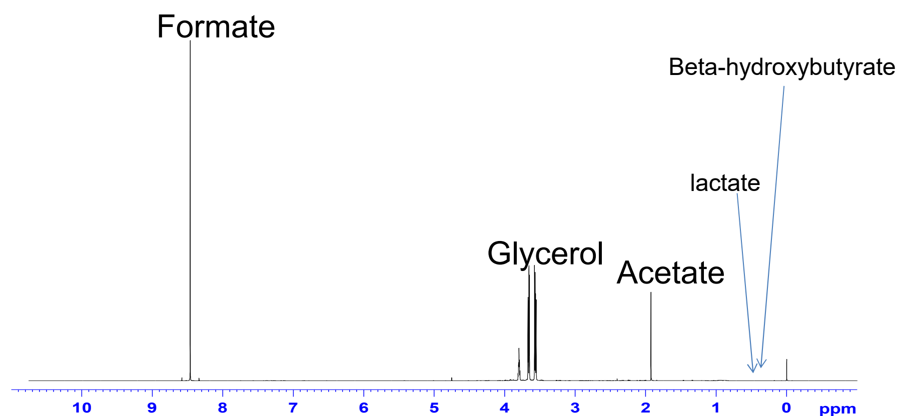
Nuclei interact with other nuclei within the molecule and between molecules

Short Chain Fatty Acids Analysis using 1H NMR



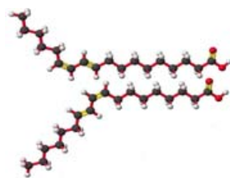
Metabolite identification in salty samples (3% salt)

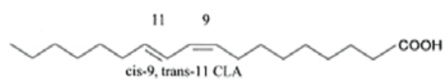
^{1}H NMR at 800 MHz

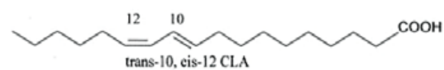


Distinguishing Isomeric Compounds : ^{13}C NMR

Conjugated Linoleic acid (CLA)

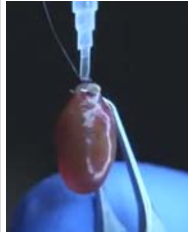


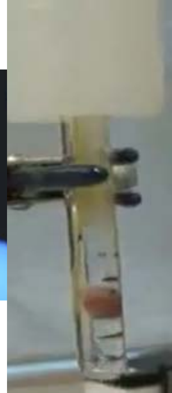




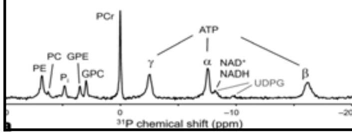
NMR of isolated organs

Mouse heart



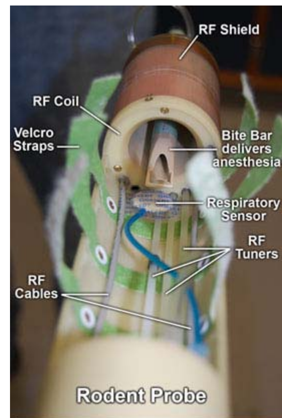


^{31}P NMR



J Vis Exp. 2010; (42): 2069.

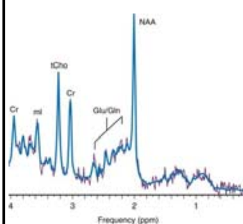
NMR of rodents



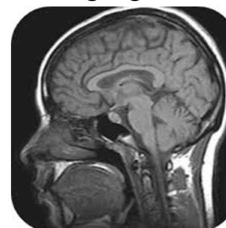
NMR of Animals

NMR of Humans

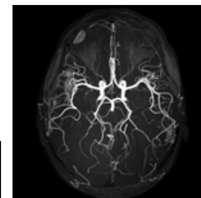
^{1}H NMR



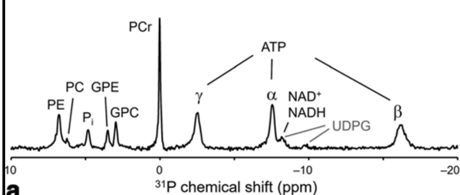
Imaging

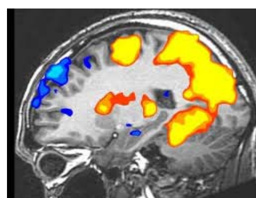


Angiography



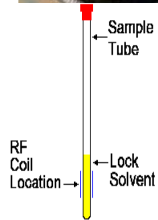
^{31}P NMR





Functional Imaging

NMR Data Pre-Processing



<u>Sample Preparation</u>

Thaw frozen sample

(1) Intact sample- Use directly for NMR

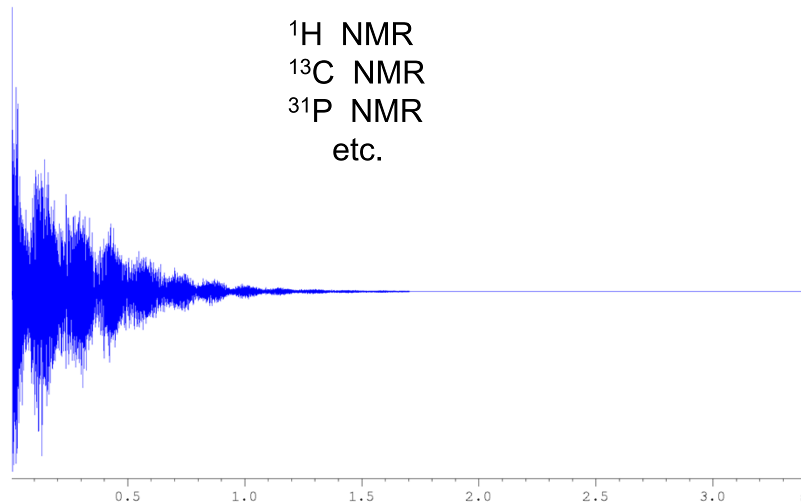
(2) Processed sample- ultrafiltration, protein precipitation (drying)

(3) Mix/dissolve with D_2O solvent/buffer (TSP/DSS)

(4) Run NMR

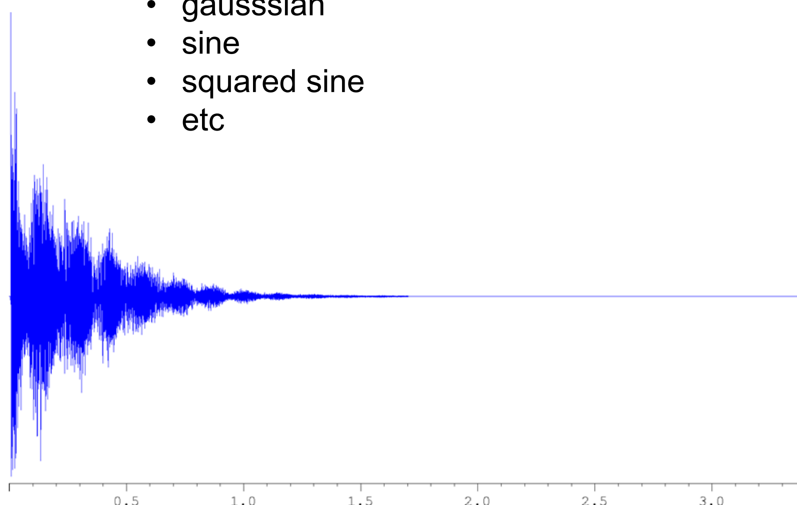
Raw data: FID (free induction decay)

^{1}H NMR
^{13}C NMR
^{31}P NMR
etc.

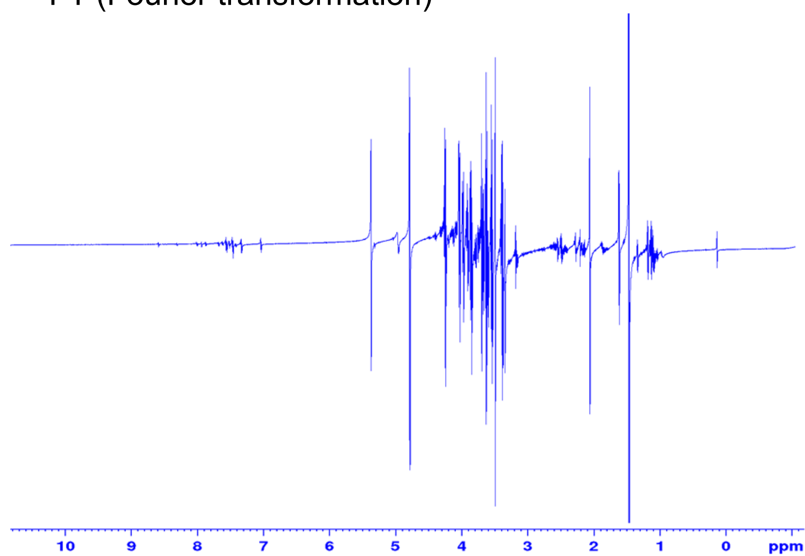


Apply window function
Smoothening or resolution enhancement

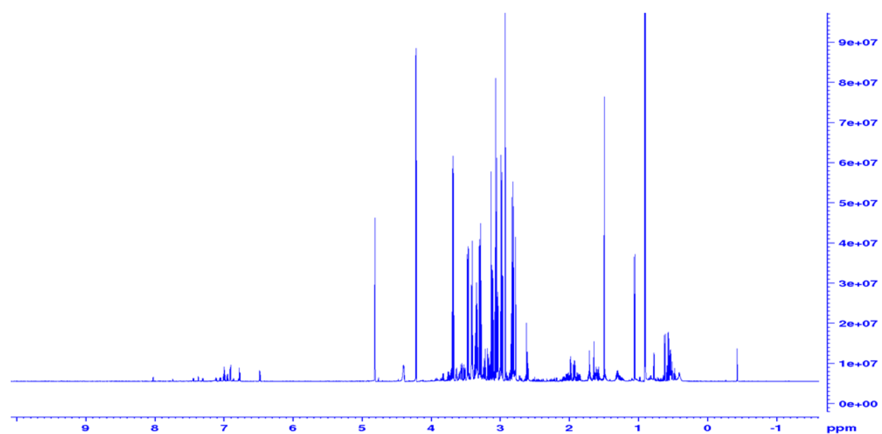
- exponential
- gausssian
- sine
- squared sine
- etc



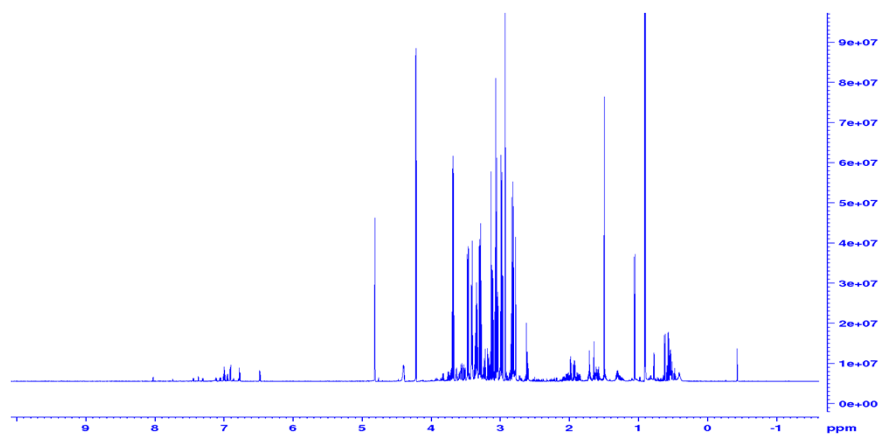
FT (Fourier transformation)

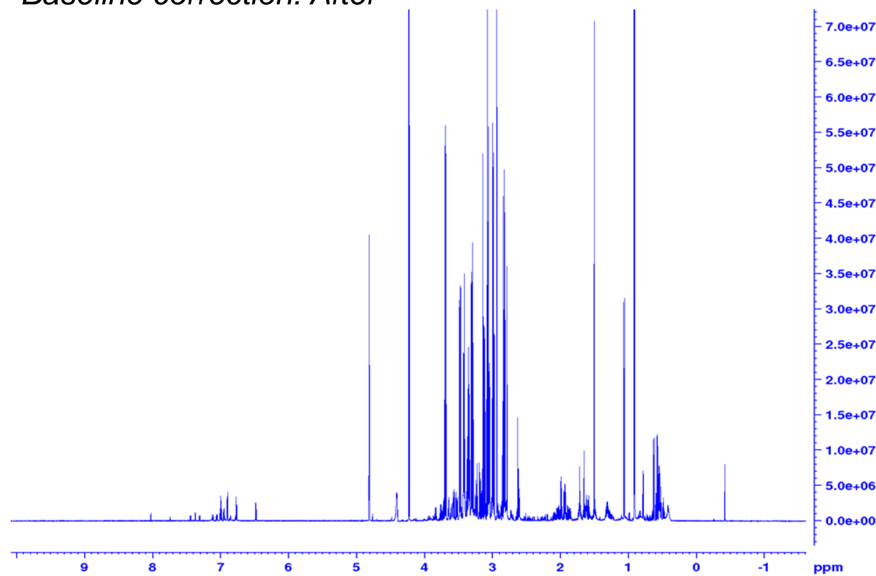


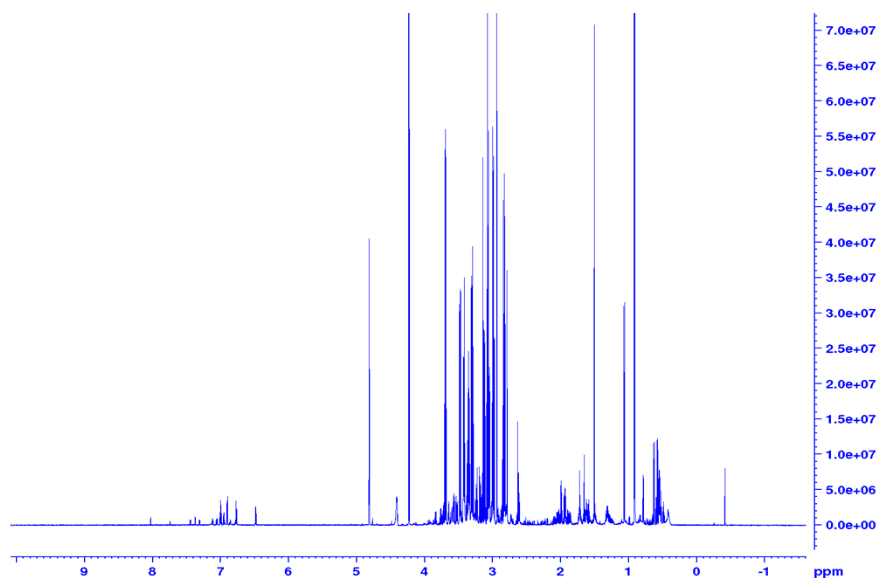
Phase correction: After



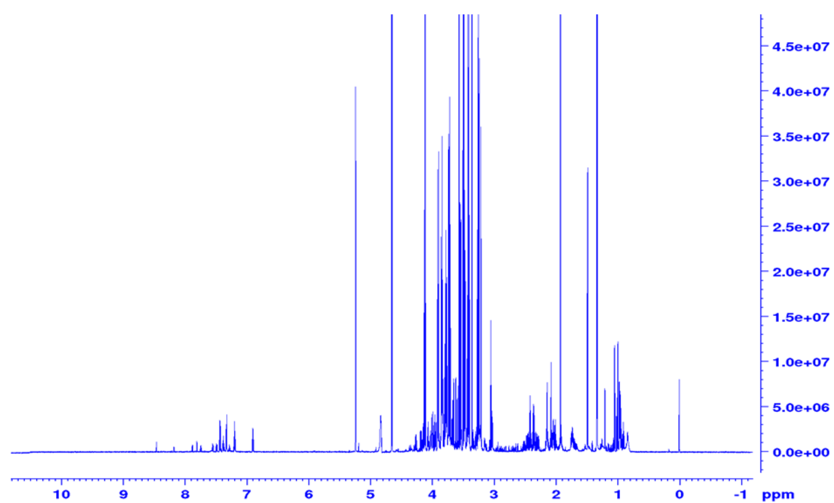
Baseline correction: Before



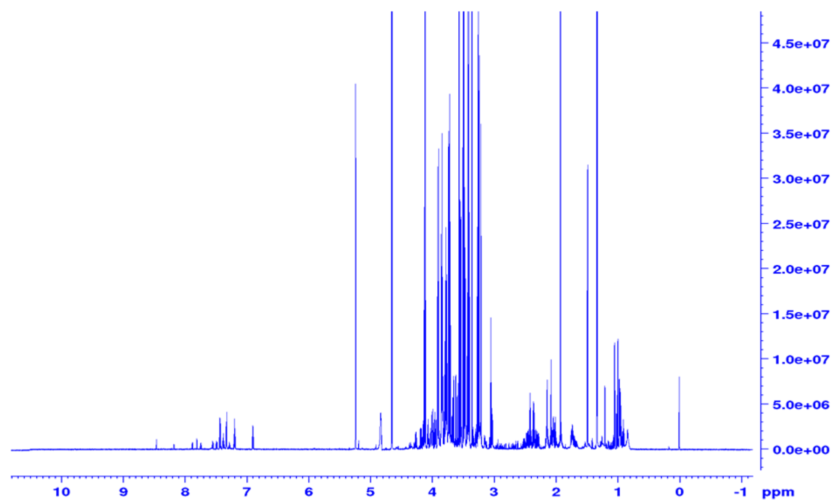

Baseline correction: After
7.0e+07
6.5e+07
6.0e+07
5.5e+07
5.0e+07
4.5e+07
4.0e+07
3.5e+07
3.0e+07
2.5e+07
2.0e+07
1.5e+07
1.0e+07
5.0e+06
0.0e+00
9
8
7
6
5
4
3
2
1
0
-1
ppm


calibration (peak alignment): Before
7.0e+07
6.5e+07
6.0e+07
5.5e+07
5.0e+07
4.5e+07
4.0e+07
3.5e+07
3.0e+07
2.5e+07
2.0e+07
1.5e+07
1.0e+07
5.0e+06
0.0e+00
9
8
7
6
5
4
3
2
1
0
-1
ppm

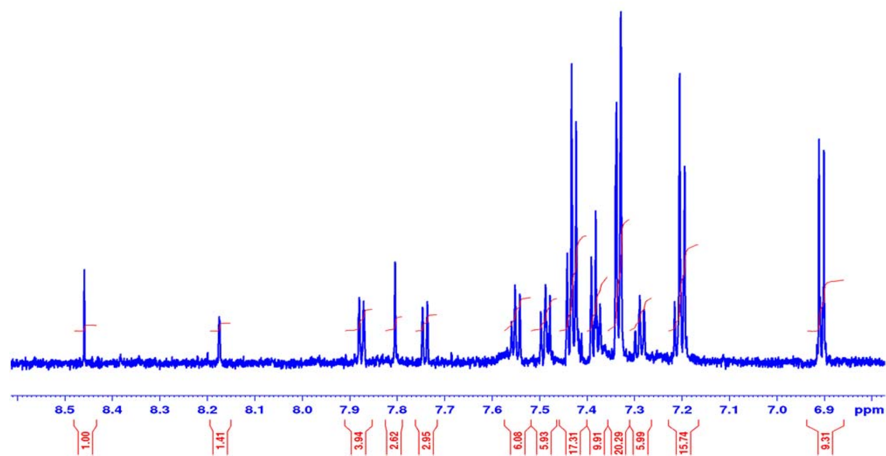
calibration (peak alignment): After



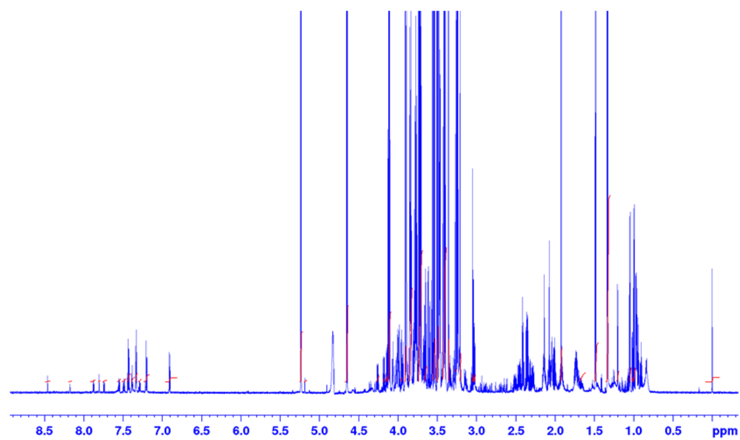
Binning

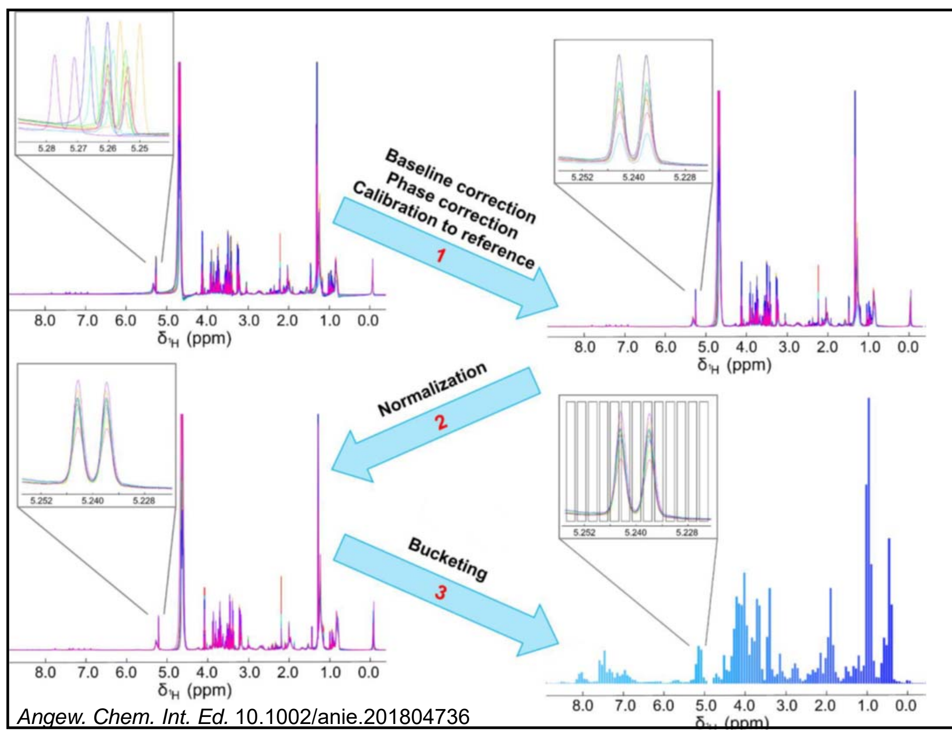


Peak integration – relative quantitation



Absolute quantitation




Baseline correction
Phase correction
Calibration to reference
1
Normalization
2
Bucketing
3
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
δ1H (ppm)
5.28 5.27 5.26 5.25
5.252 5.240 5.228
Angew. Chem. Int. Ed. 10.1002/anie.201804736