

Spectroscopy and Tumor Metabolism

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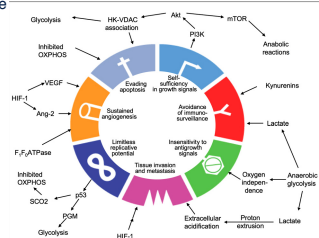
Learning Objectives

- Understand the important metabolic changes that occur in cancer and their cause.
- Awareness of the NMR approaches and pre-clinical model systems used to identify metabolic targets for patient imaging studies.
- Awareness of ^1H & hyperpolarized ^{13}C MRI metabolic imaging methods that can be used to assess tumor metabolism and the types of clinical questions that can be addressed.

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Cancer – A Metabolic Disease

- Tumors rewire metabolism to meet the bioenergetics and biosynthetic demands of cancer cell growth.
- The tumor metabolic phenotype typically includes increased consumption of glucose and glutamine, changes in the use of metabolic enzyme isoforms, and increased secretion of lactate.
- Oncogenes and tumor suppressors have key roles in cancer-associated changes in metabolism.
- Tumors also rewire metabolism for protection against reactive oxygen species and to affect their microenvironment through secreted lactate and low extracellular pH .

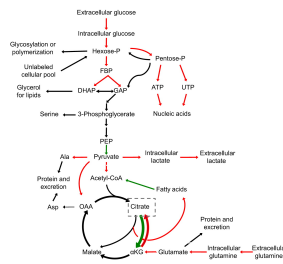


Guido Kroemer and Jacques Pouyssegur, 'Tumor Cell Metabolism: Cancer's Achilles' Heel', *Cancer Cell*, 13: 6, June 2008, 472–482.

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Glycolysis, TCA and Glutaminolysis

- There is higher flux of glucose through most metabolic pathways in tumor cells compared with normal cells. More glucose carbons are incorporated in lactate, citrate, glutamate, and metabolites involved with fatty acid synthesis, to oxidative phosphorylation in some tumors.
- Meeting all of these conditions requires increased glucose uptake which has been observed by FDG PET studies in most, but not all tumors.
- Pyruvate from glycolysis often enters a truncated TCA cycle
- This truncated TCA cycle results in a flow of metabolites out of the TCA cycle (cataplerosis) that needs to be balanced by an influx of metabolites (anaplerosis). In many cancer cells, glutamine fulfills this role



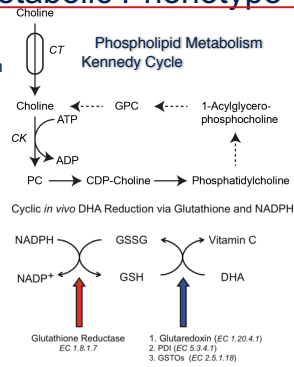
Hilary A Collier, Is Cancer a Metabolic Disease? The American Journal of Pathology, 184: 1, January 2014, 4–17.

Red lines indicates up-regulation
Green lines indicates down-regulation

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The Cancer Metabolic Phenotype

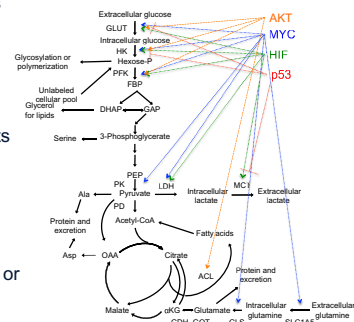
- Increased phospholipid metabolism – provides a measure of cellular proliferation – cell membrane synthesis. However, cellular degradation and other metabolic pathways can affect phospholipid metabolite levels
- Increased reducing capacity - The use of aerobic glycolysis protects tumor cells from ROS that would be generated by performing oxidative phosphorylation. Both glucose and the glutamine consumed by cancer cells can be metabolized to generate NADPH.



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Metabolic Impact - Oncogenes and Tumor Suppressor Genes

- The oncogenic molecules AKT, MYC, and hypoxia-inducible factor-1 (HIF-1) can all contribute to the cancer metabolic phenotype, whereas the tumor suppressor p53 acts to minimize the glycolytic phenotype and its loss contributes to aerobic glycolysis.
- Oncogene – gene that causes cancer – mutated or highly expressed
- Tumor suppressor gene – anticancer function



Older HA, The American Journal of Pathology 2014;184:4–17.

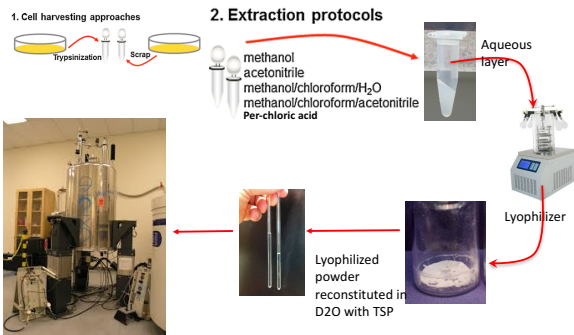
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Model systems used to study metabolomics by MR spectroscopy

- **In vitro**
 - **Cell/tissue extracts** High resolution aqueous NMR spectroscopy
 - **Intact cells** High resolution magic angle spinning NMR spectroscopy
- **Ex vivo**
 - **Patient derived tissue** Micro imaging spectrometers + perfusion systems
 - **Perfused organs** Micro imaging spectrometers + perfusion systems
- **In vivo** Requires localization via imaging gradients or surface coils

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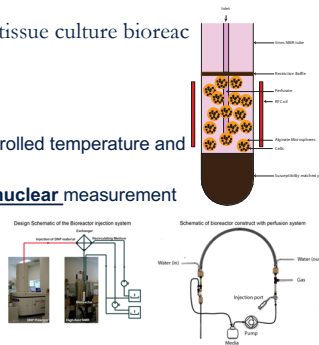
Model systems – *Ex vivo* – cell extracts



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MR compatible 3D cell/tissue culture bioreactor

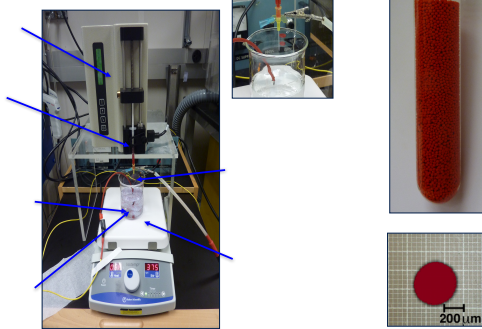
- Precision engineered
- Susceptibility matched
- Perfusion system with well controlled temperature and oxygen delivery
- Enables **serial dynamic multinuclear** measurement on the same sample
- Small sample chamber
 - Minimized use of sample
 - High mass sensitivity
 - High B₀ field homogeneity



Keshari KR, Wilson DM, Van Oostrop M, Strain R, Koelich BL, Wang ZJ, VanBrocklin HF, Pechli DM, O'Brien T, Sampath D, Carano RAD, Kurhanewicz J. Metabolic response of prostate cancer to nicotinamide phosphoribosyltransferase inhibition in a hyperpolarized MR/PET compatible bioreactor. *Prostate* doi:10.1002/proc.29006.

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Alginate microspheres

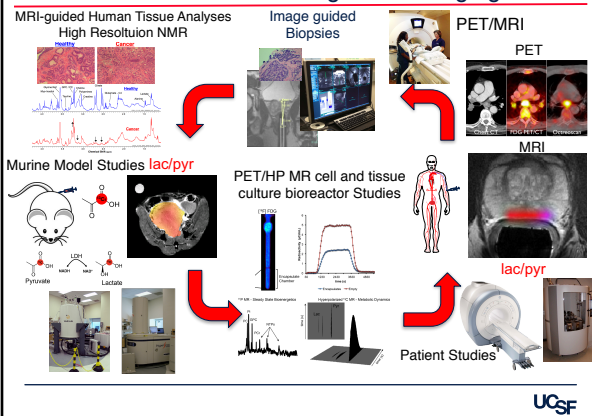


10 BioE207 Hyperpolarized MR Seminar

7/30/15

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Identification of Metabolic Targets for Imaging Studies



NMR approaches to study metabolic alterations

- Steady state metabolite profile ➡ ^1H / ^{31}P spectroscopy
- Isotopomer based flux analysis ➡ ^{13}C enriched substrate based spectroscopy
- Hyperpolarized real time enzyme kinetics ➡ DNP based ^{13}C enriched metabolites. Requires "live" cells/tissue

^{31}P spectroscopy has been used for studying bioenergetics, pH and physiology using steady state spectroscopy as well as magnetization transfer techniques

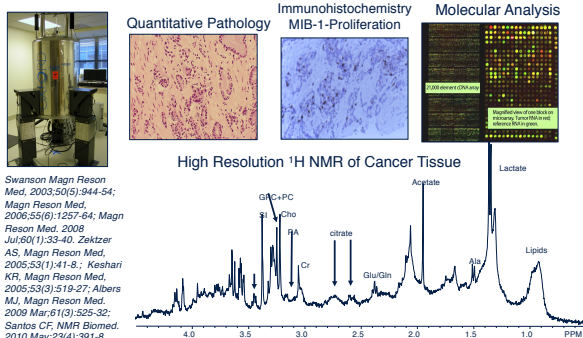
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Steady state metabolism using ^1H spectroscopy

- Models implemented in
 - Cell / Tissue extracts 1D $\rightarrow$ CPMG, presat
2D $\rightarrow$ TOCSY
 - Intact cells High resolution magic angle spinning NMR spectroscopy
 - Ex vivo
 - Patient derived tissue
- In vivo Requires localization via imaging gradients or surface coils

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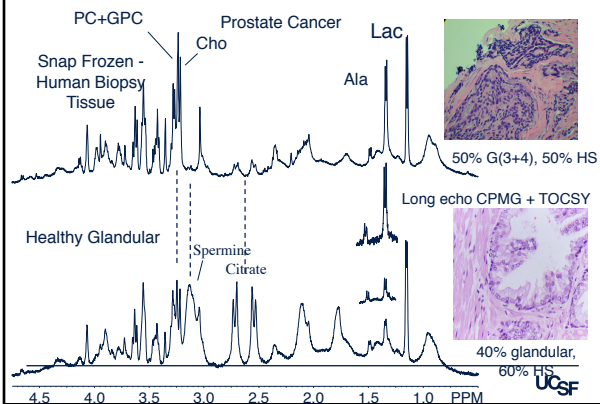
^1H NMR Spectroscopy – Extracts & Intact Tissues



High Resolution Magic Angle Spectroscopy of image targeted human biopsy sample. By spinning at the magic angle (54.7°) and using moderate spin rates (2,250 Hz), solution-like spectra can be acquired prior to pathologic, immunohistochemical, proteomic, and/or genetic analysis of the same samples.

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Metabolic Biomarkers of Prostate Cancer



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Figure 1: Correlation of phospholipid metabolites with prostate cancer pathologic grade, proliferative status and surgical stage.

The figure is divided into three main sections:

- Histological Images:** Three rows of images show prostate tissue sections stained for Choline (Ch), Phosphatidylcholine (PC), and Glycero-phosphocholine (GPC). The rows are labeled "Benign", "Gleason 3+4", and "Gleason 4+4". Each row includes a low-magnification overview image and a high-magnification inset of a specific region.
- Box Plot:** A box plot titled "GPC: PC / Cancer" shows the ratio of GPC to PC + GPC for three groups: Benign, <3+4, and >4+3. The y-axis ranges from 0 to 20. The Benign group has a ratio near 0. The <3+4 group has a median ratio around 2. The >4+3 group has a median ratio around 10. Red arrows point to the "Grade" label and the "<3+4" and ">4+3" categories.
- Kennedy Cycle Diagram:** A diagram titled "Phospholipid Metabolism Kennedy Cycle" shows the conversion of Choline to CDP-Choline and then to Phosphatidylcholine. The cycle involves the enzyme CK (Choline Kinase) and the conversion of ATP to ADP. The diagram also shows the conversion of GPC to 1-Acylglycero-phosphocholine and back to Choline.

Source: Keshari, et al. *NMR Biomed.* Correlation of phospholipid metabolites with prostate cancer pathologic grade, proliferative status and surgical stage - impact of tissue environment. *NMR Biomed.* 2011; 24(6):691-699.

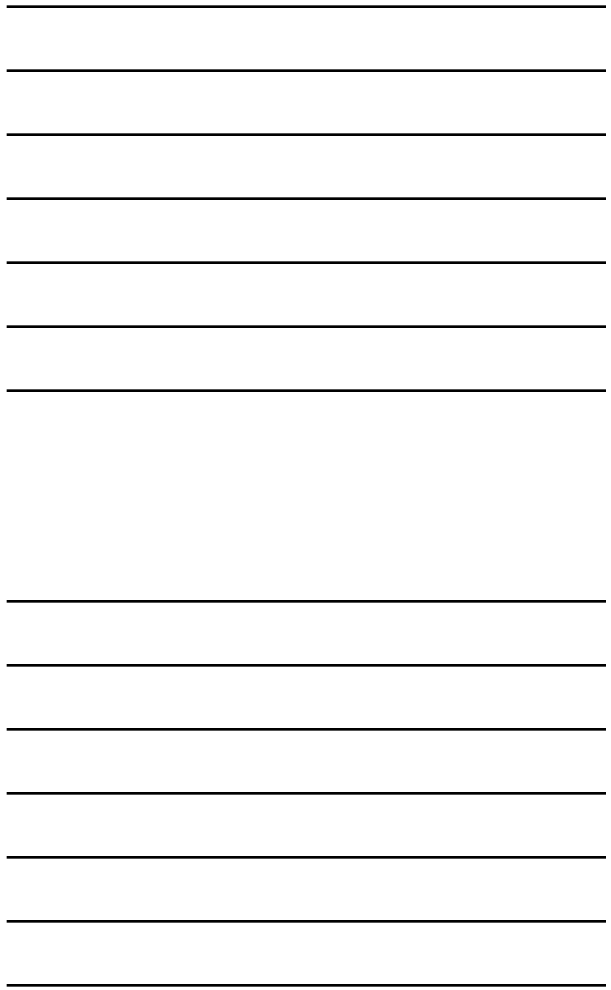


Figure 1: Discrimination of prostate cancer by metabolite ratios.

The figure illustrates the discrimination of prostate cancer using metabolite ratios, comparing Benign, Indolent cancer, and Aggressive cancer across various metabolites (Cr, Lk, Cho, PE) and metabolite ratios (PC+GPC, PC-GPC).

Top Left: MRI Scans

The top left panel shows an MRI scan of a prostate with red arrows indicating targeted biopsy sites. Below it, a grid of 12 small MRI slices shows the spatial distribution of metabolite ratios across the prostate.

Top Right: Metabolite Ratio Heatmaps

The top right panel displays heatmaps of metabolite ratios (PC+GPC and PC-GPC) for Benign, Indolent cancer, and Aggressive cancer. The heatmaps show the spatial distribution of metabolite ratios across the prostate, with color scales indicating the ratio values (e.g., 3.7 to 4.3).

Bottom Left: Histology Images

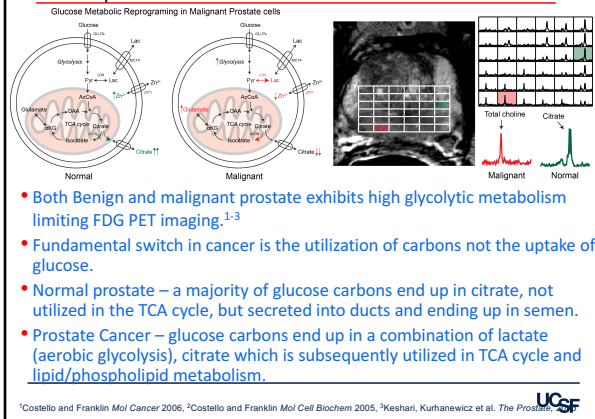
The bottom left panel shows H&E stained histology images for Benign, Indolent cancer, and Aggressive cancer, corresponding to the metabolite ratios shown in the heatmaps.

Bottom Right: Box Plots

The bottom right panel contains two box plots comparing metabolite ratios across Benign, Indolent Cancer, and Aggressive Cancer groups. The top plot shows the ratio of metabolite ratios (GPC + GPC Area Chd) and the bottom plot shows the ratio of metabolite ratios (Lactate). The box plots indicate that the ratio of metabolite ratios is significantly higher in Aggressive Cancer compared to Benign and Indolent Cancer.

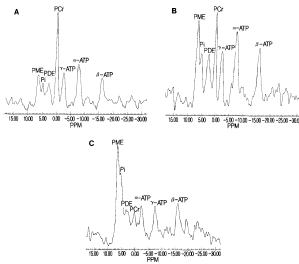
- [PC + GPC] and [lactate] obtained from post-radiation MRI targeted prostate cancer biopsies discriminated residual cancer after failed radiation therapy.
- [PC + GPC] was able to discriminate proliferating/aggressive (Ki-67 > 5%, Primary Gleason score 4) prostate cancer from indolent/dying prostate cancer after failed radiation therapy.

Unique Glucose Metabolism in Prostate Cancer



In vivo ³¹P spectroscopy in prostate

- Cancer tissue had increased PME and PDE and reduced PCr compared to normal prostate



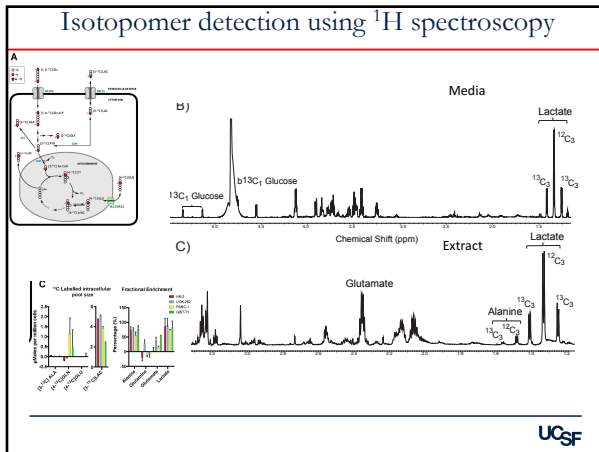
Kurhanewicz J, Thomas A, Japoda P, Weiner M, W. James, T. L., Vigneron, D. B., & Narayan, P. (1991). ³¹P spectroscopy of the human prostate gland in vivo using a transrectal probe. Magnetic Resonance in Medicine, 23(2), 404-413.

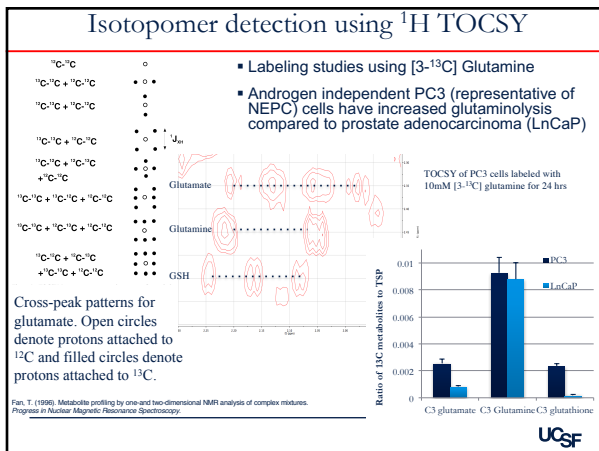
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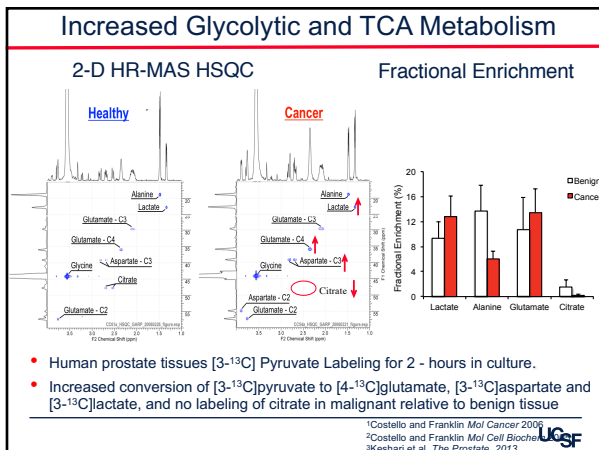
Isotopomer based metabolism using ¹H/¹³C spectroscopy

- Quantification of intracellular fluxes
- Isotopomer (Isotope + Isomer)
- Implies
 - "live" cells
 - Requires ¹³C enriched substrate
- Models implemented in
 - Cell / tissue extracts ¹H (1D, 2D), ¹H(¹³C)
¹³C
 - Intact cells
- Ex vivo
 - Organs
- In vivo
 - Requires localization via imaging gradients or surface coils

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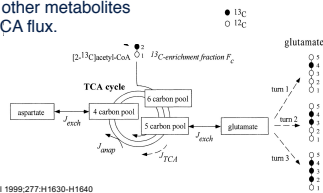






Isotomer detection using ^{13}C spectroscopy

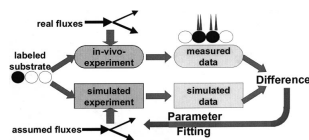
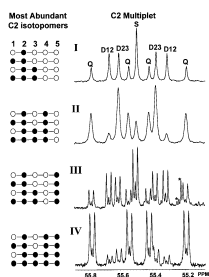
- TCA cycle is the dominant player in metabolism
 - Common pathway for oxidation
 - Provides anaplerotic substrates for biosynthesis
- Carbons flow at high rate although the TCA intermediates are at low concentration
- Glutamate a TCA intermediate in fast exchange with αKG
 - Higher concentration
 - Multiplets well resolved from other metabolites
 - Rapid exchange relative to TCA flux.



Johannes H. G. M. van Beek et al. Am J Physiol Heart Circ Physiol 1999;277:H1630-H1640

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Isotomer detection using ^{13}C spectroscopy



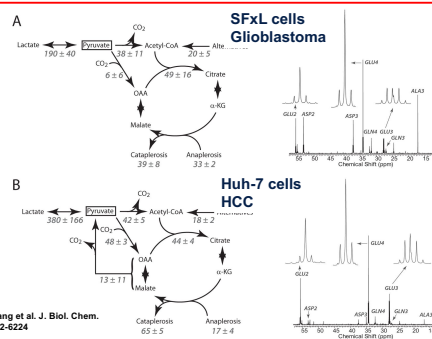
One-Bond and Multiple Bond ^{13}C - ^{13}C Couplings in Glutamate at Neutral and High pH

One-bond coupling constants	pH 6-8	pH 10-12
One bond		
J_{CH}	53.3	53.1
J_{CH_2}	34.8	34.5
J_{CH_3}	34.5	34.4
J_{CH_4}	51.3	51.3
Multiple bond		
J_{CH}	-1	-1
J_{CH_2}	2.3	2.3
J_{CH_3}	-1	-1
J_{CH_4}	-1	-1
J_{CH_5}	3.3	4.5

Carvalho, R. A., Babcock, E. E., Jeffrey, F. M., Sherry, A. D., & Malloy, C. R. (1999). Multiple bond ^{13}C - ^{13}C spin-spin coupling provides complementary information in a ^{13}C NMR isotope analysis of glutamate. *Magnetic Resonance in Medicine*, 42(1), 197-200.

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Isotomer flux analysis in tumor cells using ^{13}C spectroscopy



Chendong Yang et al. J. Biol. Chem. 2014;289:6212-6224

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