Analysis of dDNP NMR metabolic data from cancer cells

With the rise of the field of systems biology, metabolomic data have been integrated with the data for other -omic sciences, and these gigantic collections of correlated data have with the ever improving computing power, been data mined to locate biomarkers and motifs.[1] In this project the metabolic fingerprint of four prostate cancer cell lines, with different levels of aggression were analyzed. Metabolic data were obtained by incubating the cells with 13C6-d7 isotope labeled glucose, then quenching the metabolism, removing the cell debris and hyperpolarizing the metabolite extracts with dissolution Dynamic Nuclear Polarization (dDNP).

By integrating the peaks of the resulting NMR spectra, a collection of metabolic data was obtained without the need for identification of specific compounds. On this data, data mining was applied, with the aim to identify biomarkers of cancer and to classify the aggressiveness of the cancer. The illustrations below show examples of obtained NMR spectra for the different cell types (on the left) and Principal Components-Discriminant Function Analysis (PCDFA) results from the four prostate cancer cell types and a breast cancer cell line, in red, (on the right). The PC-DFA is clearly able to separate the cell types, with the most aggressive clustering together (blue and green). As dDNP MNR have been shown to be quantitative and reproducible,[2] it could be an important tool in the future for cancer diagnostics.

Kinetic Analysis of Hexose Conversion to Methyl Lactate by Sn Beta: Effects of Substrate Masking and of Water

Simple sugars bear promise as substrates for the formation of fuels and chemicals using heterogeneous catalysts in alcoholic solvents. Sn-Beta is a particularly well suited catalyst for the cleavage, isomerization and dehydration of sugars into more valuable chemicals. In order to understand these processes and save resources and time by optimising them, kinetic and mechanistic analyses are helpful. Herein, we study substrate entry into the Sn-Beta catalysed methyl lactate process using abundant hexose substrates. NMR spectroscopy is applied to show that the formation of methyl lactate occurs in two kinetic regimes for fructose, glucose and sucrose. The majority of methyl lactate is not formed from the substrate directly, but from methyl fructosides in a slow regime. At 160 °C, more than 40% of substrate carbon are masked (i.e. reversibly protected in situ) as methyl fructosides within few minutes when using hydrothermally synthesised Sn-Beta, while more than 60% methyl fructosides can be produced within few minutes using post synthetically synthesised Sn-Beta.

A significant fraction of substrate thus is masked by rapid methyl fructoside formation prior to subsequent slow release of fructose. This release is the rate limiting step in the Sn-Beta catalysed methyl lactate process, but can be accelerated by the addition of small amounts of water at the expense of maximum methyl lactate yield.
Stable isotope-resolved analysis with quantitative dissolution dynamic nuclear polarization

Metabolite profiles and their isotopomer distributions can be studied non-invasively in complex mixtures with NMR. The advent of dissolution Dynamic Nuclear Polarization (dDNP) and isotope enrichment add sensitivity and resolution to such metabolic studies. Metabolic pathways and networks can be mapped and quantified if protocols that control and exploit the ex situ signal enhancement are created. We present a sample preparation method, including cell incubation, extraction and signal enhancement, to facilitate reproducible and quantitative dDNP (qdDNP) NMR-based isotope tracer analysis. We further illustrate how qdDNP was applied to gain systematic and novel metabolic phenotypic insights into aggressive cancer cells.

General information
State: Published
Organisations: Department of Electrical Engineering, Center for Magnetic Resonance, Center for Hyperpolarization in Magnetic Resonance
Pages: 674–678
Publication date: 2018
Main Research Area: Technical/natural sciences

Publication information
Journal: Analytical Chemistry
Volume: 90
Issue number: 1
ISSN (Print): 0003-2700
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 6.24
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.08
Web of Science (2016): Indexed yes
Detecting Elusive Intermediates in Carbohydrate Conversion: A Dynamic Ensemble of Acyclic Glucose-Catalyst Complexes

The role of acyclic carbohydrates in pathways towards value-added chemicals has remained poorly characterized due to the low population of acyclic forms, and due to their instability under reaction conditions. We conduct steady-state and pre-steady state measurements by direct reaction progress monitoring with sensitivity-optimized NMR spectroscopy in the molybdate catalyzed epimerization of glucose to mannose. We detect an exchanging pool of at least five acyclic glucose-catalyst complexes under near-optimum reaction conditions. In the presence of catalyst, the acyclic glucose population increases within few seconds prior to reaching a steady state. Exchange between the acyclic intermediates increases at conditions that favor epimerization. Species accounting for less than 0.05% of total glucose can be monitored with sub-second time resolution to allow kinetic analysis of intermediate formation and catalytic conversion. Epimerization occurs 2-3 orders of magnitude-fold faster than the binding of acyclic glucose to the catalyst at near-optimum reaction conditions. The current study brings insight in to the nature of acyclic intermediate-catalyst complexes of very low population and into experimental strategies for characterizing very minor intermediates in carbohydrate conversion to value-added compounds.

General information
State: Published
Organisations: Department of Chemistry, Organic Chemistry, Center for Hyperpolarization in Magnetic Resonance, Department of Electrical Engineering, Center for Magnetic Resonance
Authors: Meier, S. (Intern), Karlsson, M. (Intern), Jensen, P. R. (Intern)
Pages: 5571-5577
Probing of biochemical pathways in clonal pancreatic β–cells by quantitative dDNP of metabolite extracts

General information
State: Published
Organisations: Department of Electrical Engineering, Center for Hyperpolarization in Magnetic Resonance, University of Copenhagen
Authors: Malinowski, R. M. (Intern), Ghiasi, S. M. (Ekstern), Mandrup-Poulsen, T. (Ekstern), Jensen, P. R. (Intern), Ardenkjær-Larsen, J. H. (Intern)
Number of pages: 1
Publication date: 2017
Event: Abstract from EUROMAR 2017, Warsaw, Poland.
Main Research Area: Technical/natural sciences
Electronic versions:
EUROMAR2017RonjaMalinowski.pdf

Relations
Activities:
Poster Presentation
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2017

Quantifying Biochemical Activities in Living Cells with $^{13}$C dDNP NMR

General information
State: Published
Organisations: Department of Electrical Engineering, Center for Hyperpolarization in Magnetic Resonance
Authors: Jensen, P. R. (Intern), Karlsson, M. (Intern), Capozzi, A. (Intern), Ardenkjær-Larsen, J. H. (Intern), Lerche, M. H. (Intern)
Quantifying Biochemical Activities in Living Cells with $^{13}$C dDNP NMR

Hyperpolarized 1-13C-1,1-bis(acetoxy(methyl)))-2,2'-cyclopropane as metabolic marker for MR

1-13C-1,1-Bis(acetoxy(methyl)))-2,2'-cyclopropane of formula (I): The compound can be hyperpolarized and used as a contrast agent in 13C Magnetic Resonance diagnostic technique (13C-MR) for the diagnosis of tumor.

Difference between Extra- and Intracellular T1 Values of Carboxylic Acids Affects the Quantitative Analysis of Cellular Kinetics by Hyperpolarized NMR

Incomplete knowledge of the longitudinal relaxation time constant (T1) leads to incorrect assumptions in quantitative kinetic models of cellular systems, studied by hyper-polarized real-time NMR. Using an assay that measures the intracellular signal of small carboxylic acids in living cells, the intracellular T1 of the carboxylic acid moiety of acetate, keto-isocaproate, pyruvate, and butyrate was determined. The intracellular T1s shown to be up to four-fold shorter than the extracellular T1. Such a large difference in T1 values between the inside and the outside of the cell has significant influence on the quantification of intracellular metabolic activity. It is expected that the significantly shorter T1 value of the carboxylic moieties inside cells is a result of macro-molecular crowding. An artificial cytosol has been prepared and applied to predict the T1 of other carboxylic acids. We demonstrate the value of this prediction tool.
Hyperpolarised Organic Phosphates as NMR Reporters of Compartmental pH

Organic phosphate metabolites contain functional groups with pH-dependent $^{13}$C chemical shift changes of adjacent quaternary carbon sites. When formed in defined cellular compartments from exogenously hyperpolarised $^{13}$C substrates, metabolites thus can yield localised pH values and correlations of organelle pH and catalytic activity.

General information
State: Published
Organisations: Center for Hyperpolarization in Magnetic Resonance, Department of Electrical Engineering, Center for Magnetic Resonance, Department of Chemistry, Organic Chemistry
Authors: Jensen, P. R. (Intern), Meier, S. (Intern)
Number of pages: 4
Pages: 2288-2291
Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information
Journal: Chemical Communications
Volume: 52
ISSN (Print): 1359-7345
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 6.03 SJR 2.555 SNIP 1.127
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.06 SJR 2.538 SNIP 1.16
Probing treatment response of glutaminolytic prostate cancer cells to natural drugs with hyperpolarized [5-13C]glutamine

General information
State: Published
Organisations: Center for Hyperpolarization in Magnetic Resonance, Department of Electrical Engineering, Center for Magnetic Resonance, Bracco Imaging
Authors: Jensen, P. R. (Intern), Canape, C. (Ekstern), Catanzaro, G. (Ekstern), Karlsson, M. (Intern), Lerche, M. H. (Intern)
Number of pages: 1
Publication date: 2016
Main Research Area: Technical/natural sciences
Electronic versions:
WMIC_poster.pdf
Source: PublicationPreSubmission
Source-ID: 130760201
Publication: Research - peer-review › Poster – Annual report year: 2017

Single-Shot-RARE for rapid 3D hyperpolarized metabolic ex vivo tissue imaging: RF-pulse design for semi-dense spectra
MRS of hyperpolarized (HP) 13C-enriched compounds is a promising method for in vivo cancer diagnosis. Sentinel lymph node ex vivo tissue sample histology used in clinical routine for breast cancer metastasis diagnosis requires time consuming sample analysis. 3D-HP-MRSI can potentially speed up the diagnosis given a sensitive marker that can be efficiently imaged in tissue after homogenous injection. The entire sample can be confined within the imaged volume giving the possibility of complete spatial non-selectivity of the radio frequency (RF) pulses in the RF pulse design with no chemical shift localization errors. Since only a few product signals are of interest for this application, a combination of under-sampled temporal encoding, frequency selective excitation and the Single-Shot-RAREsequence offers favourable SNR characteristics. Small peak separations are challenging, however, since they require narrow excitation transition-bands. We have designed a 3D-MRSI pulse sequence for hyperpolarized ex vivo sample imaging for semi-dense compound spectra (few components, relatively small separations), ultimately aimed to be used for metastasis detection in excised lymph nodes.

General information
State: Published
Organisations: Center for Hyperpolarization in Magnetic Resonance, Department of Electrical Engineering, Center for Magnetic Resonance, Department of Applied Mathematics and Computer Science, Image Analysis & Computer Graphics, Copenhagen University Hospital
Authors: Magnusson, P. (Ekstern), Jensen, P. R. (Intern), Dyrby, T. B. (Intern), Karlsson, M. (Intern), Lerche, M. H. (Intern), Hanson, L. G. (Intern)
Publication date: 2016
Event: Poster session presented at 33rd ESMRMB Annual Scientific Meeting, Vienna, Austria.
Main Research Area: Technical/natural sciences
Electronic versions:
esmrmb2016.0e049b.NORMAL.pdf
Single_Shot_RARE_for_rapid_3D_hyperpolarized_metabolic_ex_vivo_tissue_imaging_RF_pulse_design_for_semi_dense_spectra_ESMRMB_Annual_Scientific_Meeting_2016.pdf

Bibliographical note
Source: PublicationPreSubmission
Source-ID: 127578292
Publication: Research - peer-review › Poster – Annual report year: 2016

Spectroscopic approaches to resolving ambiguities of hyper-polarized NMR signals from different reaction cascades
The influx of exogenous substrates into cellular reaction cascades on the seconds time scale is directly observable by NMR spectroscopy when using nuclear spin polarization enhancement. Conventional NMR assignment spectra for the identification of reaction intermediates are not applicable in these experiments due to the non-equilibrium nature of the nuclear spin polarization enhancement. We show that ambiguities in the intracellular identification of transient reaction intermediates can be resolved by experimental schemes using site-specific isotope labelling, optimised referencing and response to external perturbations.

General information
Hyperpolarized esters as metabolic markers in MR
The invention relates to a method of Magnetic Resonance (MR) detection, in particular 13C-MR detection, by using a diagnostic medium comprising a hyperpolarized ester, in particular ethyl acetoacetate. The method comprises the detection of the MR signal of a hyperpolarized 13C carboxylic ester and of its respective hyperpolarized metabolite.

General information
State: Published
Organisations: Bracco Imaging
Authors: Jensen, P. R. (Intern), Lerche, M. H. (Intern), Karlsson, M. (Intern), Cabella, C. (Ekstern), Colombo Serra, S. (Ekstern), Miragoli, L. (Ekstern), Venturi, L. (Ekstern), Tedoldi, F. (Ekstern)
Publication date: 9 Dec 2015

Hyperpolarized 2-oxoglutarate as metabolic agent in MR
Hyperpolarized 1-13C-2-oxoglutarate as contrast agent in 13C Magnetic Resonance diagnostic technique (13C-MRI) for use in the diagnosis of cancer. In particular, upon administration of said 1-13C-2-oxoglutarate, signals of 1-13C-glutamate are detected. More in particular, different MR signals from 13C nuclei are detected and compared, said comparison being useful to determine a difference between tumor and non-tumor tissues, to determine the aggressiveness of a tumor or the efficacy of an anti-tumor therapy

General information
State: Published
Organisations: Department of Electrical Engineering, Center for Magnetic Resonance, Bracco Imaging
Authors: Jensen, P. R. (Intern), Karlsson, M. (Intern), Lerche, M. H. (Intern), Cabella, C. (Ekstern), Caminiti, L. (Ekstern), Colombo Serra, S. (Ekstern), Miragoli, L. (Ekstern), Poggi, L. (Ekstern), Venturi, L. (Ekstern), Tedoldi, F. (Ekstern)
Publication date: 2 Sep 2015

Bibliographical note
Also published as: WO2014118258 (A1) US2015374854 (A1) KR20150111353 (A) JP2016504945 (A) CN1049981256 (A)
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: EP2950826
Publication: Research › Patent – Annual report year: 2015

Hyperpolarized 2-oxoglutarate as metabolic agent in MR
Hyperpolarized 1-13C-2-oxoglutarate as contrast agent in 13C Magnetic Resonance diagnostic technique (13C-MRI) for use in the diagnosis of cancer. In particular, upon administration of said 1-13C-2-oxoglutarate, signals of 1-13C-glutamate are detected. More in particular, different MR signals from 13C nuclei are detected and compared, said comparison being useful to determine a difference between tumor and non-tumor tissues, to determine the aggressiveness of a tumor or the efficacy of an anti-tumor therapy

General information
State: Published
Organisations: Department of Electrical Engineering, Center for Magnetic Resonance, Bracco Imaging
Authors: Jensen, P. R. (Intern), Karlsson, M. (Intern), Lerche, M. H. (Intern), Cabella, C. (Ekstern), Caminiti, L. (Ekstern), Colombo Serra, S. (Ekstern), Miragoli, L. (Ekstern), Poggi, L. (Ekstern), Venturi, L. (Ekstern), Tedoldi, F. (Ekstern)
Publication date: 2 Sep 2015

Bibliographical note
Also published as: WO2014118258 (A1) US2015374854 (A1) KR20150111353 (A) JP2016504945 (A) CN1049981256 (A)
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: EP2911704
Publication: Research › Patent – Annual report year: 2015
Triarylmethyl radicals.
New radical compounds, useful in the field of MRI imaging of formula (I). The radical compounds are in particular new triarylmethyl ("trityl") radicals which can be used as polarizing agents for polarizing a molecule in the DNP process.

**General information**
State: Published
Organisations: Bracco Imaging
Authors: Karlsson, M. (Intern), Napolitano, R. (Ekstern), Visigalli, M. (Ekstern), Lerche, M. H. (Intern), Jensen, P. R. (Intern), Tedoldi, F. (Ekstern)
Publication date: 20 May 2015

**Publication information**
IPC: G01R 33/62 A1
Patent number: EP2872520
Date: 20/05/2015
Priority date: 04/07/2013
Priority number: WO2013EP64121
Original language: English
Electronic versions:
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: EP2872520
Publication: Research › Patent – Annual report year: 2015

**Intra-operative cancer diagnosis based on a hyperpolarized marker**
The present invention is concerned with an in vitro method of diagnosing cancer in a tissue sample, wherein said tissue sample is obtained from a patient undergoing cancer surgery. The method described herein is based on a hyperpolarized marker, which is contacted with the tissue sample, and an NMR spectrum and/or an MR image obtained of the tissue sample after having been contacted with the hyperpolarized marker.

**General information**
State: Published
Organisations: Albeda Research ApS
Authors: Lerche, M. H. (Intern), Karlsson, M. (Intern), Jensen, P. R. (Intern)
Publication date: 18 Mar 2015

**Publication information**
IPC: G01N 33/574 A1
Patent number: EP2847591
Date: 18/03/2015
Priority date: 07/05/2013
Priority number: WO2013EP59481
Original language: English
Electronic versions:
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: EP2847591
Publication: Research › Patent – Annual report year: 2015

**Hyperpolarized amino acids**
Method for manufacturing a hyperpolarized amino acid, in particular glutamine, which substantially limits the formation of by-products, with respect to conventional aqueous preparations of sodium hydroxide with amino acids. The amino acid is in particular admixed with the hydroxide in the substantial absence of water and the dry mixture is dissolved in an anhydrous solvent in the presence of a polarizing agent. The obtained mixture is then subjected to a DNP process and can be used in metabolic MR imaging.

**General information**
State: Published
Organisations: Bracco Imaging
Authors: Karlsson, M. (Intern), Lerche, M. H. (Intern), Jensen, P. R. (Intern)
Publication date: 11 Feb 2015
Hyperpolarized [1,3-C-13(2)]ethyl acetoacetate is a novel diagnostic metabolic marker of liver cancer

An increased prevalence of liver diseases such as hepatitis C and nonalcoholic fatty liver results in an augmented incidence of the most common form of liver cancer, hepatocellular carcinoma (HCC). HCC is most often found in the cirrhotic liver and it can therefore be challenging to rely on anatomical information alone when diagnosing HCC. Valuable information on specific cellular metabolism can be obtained with high sensitivity thanks to an emerging magnetic resonance (MR) technique that uses C-13 labeled hyperpolarized molecules. Our interest was to explore potential new high contrast metabolic markers of HCC using hyperpolarized C-13-MR. This work led to the identification of a class of substrates, low molecular weight ethyl-esters, which showed high specificity for carboxyl esterases and proved in many cases to possess good properties for signal enhancement. In particular, hyperpolarized [1,3-C-13(2)]ethyl acetoacetate (EAA) was shown to provide a metabolic fingerprint of HCC. Using this substrate a liver cancer implanted in rats was diagnosed as a consequence of an approximate to 4 times higher metabolic substrate-to-product ratio than in the surrounding healthy tissue, (p=0.009). Unregulated cellular uptake as well as cosubstrate independent enzymatic conversion of EAA, made this substrate highly useful as a hyperpolarized C-13-MR marker. This could be appreciated by the signal-to-noise (SNR) obtained from EAA, which was comparable to the SNR reported in a literature liver cancer study with state-of-the-art hyperpolarized substrate, [1-C-13]pyruvate. Also, the contrast-to-noise (CNR) in the EAA based metabolic ratio images was significantly improved compared with the CNR in equivalent images reported using [1-C-13]pyruvate. What's New? An emerging approach to metabolic imaging in cancer is based on C-13-hyperpolarized magnetic resonance (MR) spectroscopy. But metabolic markers specific to cancer must be identified to realize its promise. Here, metabolic conversion of hyperpolarized esters was explored as a potential means of detecting hepatocellular carcinoma (HCC). Hyperpolarized [1,3-C-13(2)]ethyl acetoacetate (EAA) was found to be an exceptionally good substrate for carboxyl esterase-1, concentrations and activities of which are altered in cancer cells. Metabolic conversion of EAA as a substrate for C-13-hyperpolarized MR significantly enhanced the detection of implanted liver cancer in rats.
General information
State: Published
Authors: Lerche, M. H. (Intern), Jensen, P. R. (Intern), Karlsson, M. (Intern), Meier, S. (Intern)
Pages: 119–132
Publication date: 2015
Main Research Area: Technical/natural sciences

Publication information
Journal: Analytical Chemistry
Volume: 87
ISSN (Print): 0003-2700
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 6.24
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 6
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Probing treatment response of glutaminolytic prostate cancer cells to natural drugs with hyperpolarized \([5-C-13]\)glutamine: Glutaminolysis to Measure Drug Response in Cancer Cells

Purpose
The correlation between glutamine metabolism and oncogene expression in cancers has led to a renewed interest in the role of glutamine in cancer cell survival. Hyperpolarized \([5-C-13]\)glutamine is evaluated as a potential biomarker for noninvasive metabolic measurements of drug response in prostate cancer cells.

Methods
Hyperpolarized \([5-C-13]\)glutamine is used to measure glutamine metabolism in two prostate cancer cell lines (PC3 and DU145) before and after treatment with the two natural anticancer drugs resveratrol and sulforaphane. An invasive biochemical assay simulating the hyperpolarized experiment is used to independently quantify glutamine metabolism.

Results
Glutamine metabolism is found to be 4 times higher in the more glutaminolytic DU145 cells compared with PC3 cells under proliferating growth conditions by using hyperpolarized \([5-C-13]\)glutamine as a noninvasive probe. A significant decrease in glutamine metabolism occurs upon apoptotic response to treatment with resveratrol and sulforaphane.

Conclusion
Hyperpolarized NMR using \([5-C-13]\)glutamine as a probe permits the noninvasive observation of glutaminolysis in different cell lines and under different treatment conditions. Hyperpolarized \([5-C-13]\)glutamine metabolism thus is a promising biomarker for the noninvasive detection of tumor response to treatment, as it directly monitors one of the hallmarks in cancer metabolism - glutaminolysis - in living cells.


General information
State: Published
Organisations: University of Torino, Albeda Research ApS, Bracco Imaging
Authors: Canape, C. (Ekstern), Catanzaro, G. (Ekstern), Terreno, E. (Ekstern), Karlsson, M. (Intern), Lerche, M. H. (Intern), Jensen, P. R. (Intern)
Pages: 2296-2305
Publication date: 2015
Main Research Area: Technical/natural sciences
Process for the preparation of hyperpolarized derivatives for use in MRI analysis

The present invention relates to a process for the preparation of aqueous solutions of hyperpolarized carboxylic organic acids ready for use in in-vivo MR diagnostic imaging, and the use of the corresponding anhydrides or esters as glass-forming agents.

General information
State: Published
Organisations: Bracco Imaging
Authors: Aime, S. (Ekstern), Battista Giovenzana, G. (Ekstern), Tedoldi, F. (Ekstern), Jensen, P. R. (Intern), Karlsson, M. (Intern), Lerche, M. H. (Intern), Colombo Serra, S. (Ekstern)
Publication date: 20 Aug 2014

Publication information
IPC: A61K 49/10 A I
Patent number: EP2766050
Date: 20/08/2014
Priority date: 11/10/2012
Priority number: WO2012EP70187
Original language: English
Electronic versions:

Bibliographical note
Also published as: WO2013053839 (A1) US2014257085 (A1)
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: EP2766050
Publication: Research - peer-review › Patent – Annual report year: 2014

Hyperpolarized NMR Probes for Biological Assays

During the last decade, the development of nuclear spin polarization enhanced (hyperpolarized) molecular probes has opened up new opportunities for studying the inner workings of living cells in real time. The hyperpolarized probes are produced ex situ, introduced into biological systems and detected with high sensitivity and contrast against background signals using high resolution NMR spectroscopy. A variety of natural, derivatized and designed hyperpolarized probes has emerged for diverse biological studies including assays of intracellular reaction progression, pathway kinetics, probe uptake and export, pH, redox state, reactive oxygen species, ion concentrations, drug efficacy or oncogenic signaling. These probes are readily used directly under natural conditions in biofluids and are often directly developed and optimized for cellular assays, thus leaving little doubt about their specificity and utility under biologically relevant conditions. Hyperpolarized molecular probes for biological NMR spectroscopy enable the unbiased detection of complex processes by virtue of the high spectral resolution, structural specificity and quantifiability of NMR signals. Here, we provide a survey of strategies used for the selection, design and use of hyperpolarized NMR probes in biological assays, and describe current limitations and developments.

General information
Non-invasive in-cell determination of free cytosolic [NAD+] /[NADH] ratios using hyperpolarized glucose show large variations in metabolic phenotypes

Background: Free cytosolic [NAD+] /[NADH] ratio maintains cellular redox homeostasis and is a cellular metabolic readout.

Results: Pyruvate/lactate ratios show distinct metabolic phenotypes and are used to derive free cytosolic [NAD+] /[NADH] ratios.

Conclusion: Determination of free cytosolic [NAD+] /[NADH] ratios using hyperpolarized glucose is applicable to a wide selection of cell types. Significance: This metabolic phenotyping may be a crucial tool to understand pathologies, and to diagnose and measure effects of therapies. © 2014 by The American Society for Biochemistry and Molecular Biology, Inc.
Structural basis and dynamics of multidrug recognition in a minimal bacterial multidrug resistance system

TipA is a transcriptional regulator found in diverse bacteria. It constitutes a minimal autoregulated multidrug resistance system against numerous thiopeptide antibiotics. Here we report the structures of its drug-binding domain TipAS in complexes with promothiocin A and nosiheptide, and a model of the thiostrepton complex. Drug binding induces a large...
transition from a partially unfolded to a globin-like structure. The structures rationalize the mechanism of promiscuous, yet specific, drug recognition: (i) a four-ring motif present in all known TipA-inducing antibiotics is recognized specifically by conserved TipAS amino acids; and (ii) the variable part of the antibiotic is accommodated within a flexible cleft that rigidifies upon drug binding. Remarkably, the identified four-ring motif is also the major interacting part of the antibiotic with the ribosome. Hence the TipA multidrug resistance mechanism is directed against the same chemical motif that inhibits protein synthesis. The observed identity of chemical motifs responsible for antibiotic function and resistance may be a general principle and could help to better define new leads for antibiotics.
Composition comprising acetic anhydride and a gadolinium complex, and method for the use in hyperpolarisation MRI analysis.

The present invention generally relates to a composition comprising acetic anhydride, a DNP agent and a gadolinium complex and its use for the preparation of hyperpolarised imaging agent for MR diagnostic analysis.

General information
State: Published
Organisations: Bracco Imaging
Authors: Lerche, M. H. (Intern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Colombo Serra, S. (Ekstern), Visigalli, M. (Ekstern), Aime, S. (Ekstern), Tedoldi, F. (Ekstern)
Publication date: 13 Jun 2013

Publication information
IPC: A61K 49/ 10 A I
Patent number: WO2013083535
Date: 13/06/2013
Priority date: 05/12/2011
Priority number: EP20110191872

10.1073/pnas.1412070111
Source: FindIt
Source-ID: 273053665
Publication: Research - peer-review › Journal article – Annual report year: 2014
Process for preparing hyperpolarized substrates and method for MRI
The present invention generally relates to a process for the preparation of aqueous solutions of hyperpolarized molecules ready for use in in-vivo MR diagnostic imaging, the use thereof as MRI contrast agent in investigation methods for producing diagnostic MR images of a human or non-human animal body organ, region or tissue.

In vivo and in vitro liver cancer metabolism observed with hyperpolarized [5-C-13]glutamine
Glutamine metabolism is, with its many links to oncogene expression, considered a crucial step in cancer metabolism and it is thereby a key target for alteration in cancer development. In particular, strong correlations have been reported between oncogene expression and expression and activity of the enzyme glutaminase. This mitochondrial enzyme, which is responsible for the deamidation of glutamine to form glutamate, is overexpressed in many tumour tissues. In animal models, glutaminase expression is correlated with tumour growth rate and it is readily possible to limit tumour growth by suppression of glutaminase activity. In principle, hyperpolarized C-13 MR spectroscopy can provide insight to glutamine metabolism and should hence be a valuable tool to study changes in glutaminase activity as tumours progress. However, no such successful in vivo studies have been reported, even though several good biological models have been tested. This may, at least partly, be due to problems in preparing glutamine for hyperpolarization. This paper reports a new and improved preparation of hyperpolarized [5-C-13]glutamine, which provides a highly sensitive C-13 MR marker. With this preparation of hyperpolarized [5-C-13]glutamine, glutaminase activity in vivo in a rat liver tumour was investigated. Moreover, this marker was also used to measure response to drug treatment in vitro in cancer cells. These examples of [5-C-13]glutamine used in tumour models warrant the new preparation to allow metabolic studies with this conditionally essential amino acid. (C) 2013 Elsevier Inc. All rights reserved.
Real-Time DNP NMR Observations of Acetic Acid Uptake, Intracellular Acidification, and of Consequences for Glycolysis and Alcoholic Fermentation in Yeast

Uptake and upshot in vivo: Straightforward methods that permit the real-time observation of organic acid influx, intracellular acidification, and concomitant effects on cellular-reaction networks are crucial for improved bioprocess monitoring and control (see scheme). Herein, dynamic nuclear polarization (DNP) NMR is used to observe acetate influx, ensuing intracellular acidification and the metabolic consequences on alcoholic fermentation and glycolysis in living cells.

General information
State: Published
Authors: Jensen, P. R. (Intern), Karlsson, M. (Intern), Lerche, M. H. (Intern), Meier, S. (Intern)
Pages: 13288 – 13293
Publication date: 2013
Main Research Area: Technical/natural sciences

Publication information
Journal: Chemistry: A European Journal
Volume: 19
ISSN (Print): 0947-6539
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.9 SJR 2.265 SNIP 1.02
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 5.03 SJR 2.352 SNIP 1.068
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.461 SNIP 1.195 CiteScore 4.99
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.526 SNIP 1.222 CiteScore 5.51
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.643 SNIP 1.239 CiteScore 5.68
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.935 SNIP 1.291 CiteScore 5.55
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.902 SNIP 1.319 CiteScore 5.46
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
WhiB7, an Fe-S-dependent Transcription Factor That Activates Species-specific Repertoires of Drug Resistance Determinants in Actinobacteria.

WhiB-like (Wbl) proteins are well known for their diverse roles in actinobacterial morphogenesis, cell division, virulence, primary and secondary metabolism, and intrinsic antibiotic resistance. Gene disruption experiments showed that three different Actinobacteria (Mycobacterium smegmatis, Streptomyces lividans, and Rhodococcus jostii) each exhibited a different whiB7-dependent resistance profile. Heterologous expression of whiB7 genes showed these resistance profiles reflected the host's repertoire of endogenous whiB7-dependent genes. Transcriptional activation of two resistance genes in the whiB7 regulon, tap (a multidrug transporter) and erm(37) (a ribosomal methyltransferase), required interaction of WhiB7 with their promoters. Furthermore, heterologous expression of tap genes isolated from Mycobacterium species demonstrated that divergencies in drug specificity of homologous structural proteins contribute to the variation of WhiB7-dependent drug resistance. WhiB7 has a specific tryptophan/glycine-rich region and four conserved cysteine residues; it also has a peptide sequence (AT-hook) at its C terminus that binds AT-rich DNA sequence motifs upstream of the promoters it activates. Targeted mutagenesis showed that these motifs were required to provide antibiotic resistance in vivo. Anaerobically purified WhiB7 from S. lividans was dimeric and contained 2.1 ± 0.3 and 2.2 ± 0.3 mol of iron and sulfur, respectively, per protomer (consistent with the presence of a 2Fe-2S cluster). However, the properties of the dimer's absorption spectrum were most consistent with the presence of an oxygen-labile 4Fe-4S cluster, suggesting 50% occupancy. These data provide the first insights into WhiB7 iron-sulfur clusters as they exist in vivo, a major unresolved issue in studies of Wbl proteins.

General information
State: Published
Organisations: University of Basel, Case Western Reserve University, University of British Columbia
Authors: Ramón-García, S. (Ekstern), Ng, C. (Ekstern), Jensen, P. R. (Intern), Dosanjh, M. (Ekstern), Burian, J. (Ekstern), Morris, R. P. (Ekstern), Folcher, M. (Ekstern), Eltis, L. D. (Ekstern), Grzesiek, S. (Ekstern), Nguyen, L. (Ekstern), Thompson, C. J. (Ekstern)
Pages: 34514-34528
Development of Dissolution DNP-MR Substrates for Metabolic Research

Dissolution dynamic nuclear polarization (DNP) provides a broadly applicable and rather simple means of developing probes for the real-time molecular imaging of cellular functions in vivo. The development of novel dissolution DNP substrate formulations is only rewarding for substrates that yield detectable metabolism within few minutes. In addition, in vivo preparations usually require amorphous samples at molar substrate concentrations for an efficient and reproducible DNP step with sufficient material. The composition ranges of novel substrate preparations need to be established experimentally owing to the solute’s impact on vitrification behavior. Here, we describe simple rationales employed in the development of novel substrate preparations for dissolution DNP-magnetic resonance. Solution state substrate polarizations between 10 and 40 % have been obtained for ~40 metabolic substrates in highly concentrated preparations that yield physiologically tolerable solutions with sufficient T1 for in vivo nuclear magnetic resonance. Substrate metabolism is observed for novel in vivo substrates such as 3-hydroxybutyrate and aspartate.
Direct Observation of Metabolic Differences in Living Escherichia Coli Strains K-12 and BL21

General information
State: Published
Organisations: Carlsberg Laboratory, Albeda Research ApS
Authors: Meier, S. (Intern), Jensen, P. R. (Intern), Duus, J. Ø. (Intern)
Number of pages: 3
Pages: 308-310
Publication date: 2012
Main Research Area: Technical/natural sciences

Publication information
Journal: ChemBioChem
Volume: 13
Issue number: 2
ISSN (Print): 1439-4227
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.64 SJR 1.407 SNIP 0.721
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.64 SJR 1.283 SNIP 0.735
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.268 SNIP 0.749 CiteScore 2.77
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.392 SNIP 0.85 CiteScore 2.88
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.634 SNIP 0.847 CiteScore 3.15
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.874 SNIP 0.901 CiteScore 3.49
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.921 SNIP 0.952 CiteScore 3.59
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.981 SNIP 0.929
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.928 SNIP 0.927
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.989 SNIP 0.867
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.048 SNIP 0.986
Scopus rating (2006): SJR 1.938 SNIP 0.956
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.877 SNIP 0.953
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.734 SNIP 1.026
Scopus rating (2003): SJR 1.662 SNIP 1.076
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 7.15 SNIP 1.347
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.887 SNIP 0.228

Original language: English
Escherichia coli K12, Glucose, Phenotype, Sequence Alignment, Species Specificity, IY9XDZ35W2 Glucose, glucose, recombinant protein, article, bacterial metabolism, bacterial strain, biochemistry, biotechnology, carbon nuclear magnetic resonance, Escherichia coli, gene identification, gene overexpression, gene product, gene sequence, genetic difference, glucose metabolism, glycolysis, hydrolysis, hyperpolarization, molecular cloning, nonhuman, nuclear magnetic resonance spectroscopy, priority journal, protein expression, signal transduction, Gene function, Hyperpolarization, In vivo NMR, Metabolism, Pentose phosphate pathway, cell growth, cellular physiology, genome, metabolic difference, phenotype, UV irradiation, Facultatively Anaerobic Gram-Negative Rods Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Enterobacteriaceae [06702] Escherichia coli species strain-BL21, strain-K-12, Escherichia coli ybhE gene [Enterobacteriaceae], 2,4-dinitrophenol, 6-phosphogluconate 921-62-0, 6-phosphogluconate dehydrogenase 9073-95-4 EC 1.1.1.44, 6-phosphogluconolactonase 37278-45-8 EC 3.1.1.31, acetyl-CoA 72-89-9, delta-6-phosphogluconolactone, dihydroxyacetone phosphate 57-04-5, gamma-6-phosphogluconolactone, gluconate 608-59-3, gluconic acid 528-95-4, glucose 58367-01-4 metabolism, glucose-6-phosphate 56-73-5, glucose-6-phosphate dehydrogenase 9001-40-5 EC 1.1.1.49, lactone II, pentose phosphate pathway, recombinant proteins expression, ribulose-5-phosphate 4151-19-3, 02502, Cytology - General, 03502, Genetics - General, 10060, Biochemistry studies - General, 10062, Biochemistry studies - Nucleic acids, purines and pyrimidines, 10064, Biochemistry studies - Proteins, peptides and amino acids, 10068, Biochemistry studies - Carbohydrates, 10802, Enzymes - General and comparative studies: coenzymes, 13002, Metabolism - General metabolism and metabolic pathways, 30500, Morphology and cytology of bacteria, 31000, Physiology and biochemistry of bacteria, 31500, Genetics of bacteria and viruses, Biochemistry and Molecular Biophysics, liquid-state nuclear magnetic resonance spectroscopy laboratory techniques, spectrum analysis techniques, metabolic engineering laboratory techniques, Cell Biology, Methods and Techniques, Molecular Genetics, BIOCHEMISTRY, CHEMISTRY, CENTRAL CARBON METABOLISM, ENCODING 6-PHOSPHOGLUCONOLACTONASE, GENOME SEQUENCES, NMR, BL21(DE3), PROTEINS, MUTANTS, REL606, GENE, gene function, in vivo NMR, metabolism

DOIs:
10.1002/cbic.201100654
Source: FindIt
Source-ID: 191178492
Publication: Research - peer-review › Journal article – Annual report year: 2012
Sulfite Action in Glycolytic Inhibition: In Vivo Real-Time Observation by Hyperpolarized 13C NMR Spectroscopy

Detecting the molecular targets of xenobiotic substances in vivo poses a considerable analytical challenge. Here, we describe the use of an NMR-based tracer methodology for the instantaneous in vivo observation of sulfur(IV) action on cellular metabolism. Specifically, we find that glycolytic flux is directed towards sulfite adducts of dihydroxyacetone phosphate and pyruvate as off-pathway intermediates that obstruct glycolytic flux. In particular, the pyruvatesulfite association hinders the formation of downstream metabolites. The apparent in vivo association constant of pyruvate and sulfite agrees with the apparent inhibition constant of CO2 formation, thus supporting the importance of pyruvate interception in disturbing central metabolism and inhibiting NAD regeneration.
Metabolic pathway visualization in living yeast by DNP-NMR

Central carbon metabolism of living Saccharomyces cerevisiae is visualized by DNP-NMR. Experiments are conducted as real time assays that detect metabolic bottlenecks, pathway use, reversibility of reactions and reaction mechanisms in vivo with subsecond time resolution.

General information
State: Published
Organisations: Albeda Research ApS, Carlsberg Laboratory
Authors: Meier, S. (Intern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Lerche, M. H. (Intern), Duus, J. Ø. (Intern)
Pages: 2834-2836
Publication date: 2011
Main Research Area: Technical/natural sciences

Publication information
Journal: Molecular BioSystems
Volume: 7
Issue number: 10
ISSN (Print): 1742-206X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.789 SJR 1.084 CiteScore 2.75
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.85 SJR 1.171 SNIP 0.753
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Quantitative dynamic nuclear polarization-NMR on blood plasma for assays of drug metabolism

Analytical platforms for the fast detection, identification and quantification of circulating drugs with a narrow therapeutic range are vital in clinical pharmacology. As a result of low drug concentrations, analytical tools need to provide high sensitivity and specificity. Dynamic nuclear polarization-NMR (DNP-NMR) in the form of the hyperpolarization–dissolution method should afford the sensitivity and spectral resolution for the direct detection and quantification of numerous isotopically labeled circulating drugs and their metabolites in single liquid-state NMR transients. This study explores the capability of quantitative in vitro DNP-NMR to assay drug metabolites in blood plasma. The lower limit of detection for the anti-epileptic drug 13C-carbamazepine and its pharmacologically active metabolite 13C-carbamazepine-10,11-epoxide is 0.08 µg/mL in rabbit blood plasma analyzed by single-scan 13C DNP-NMR. An internal standard is used for the accurate quantification of drug and metabolite. Comparison of quantitative DNP-NMR data with an established analytical method (liquid chromatography-mass spectrometry) yields a Pearson correlation coefficient r of 0.99. Notably, all DNP-NMR determinations were performed without analyte derivatization or sample purification other than plasma protein precipitation. Quantitative DNP-NMR is an emerging methodology which requires little sample preparation and yields quantitative data with high sensitivity for therapeutic drug monitoring. Copyright © 2010 John Wiley & Sons, Ltd.

Keyword: Blood plasma, DNP-NMR, Carbamazepine, Drug metabolism, C NMR, Therapeutic drug monitoring

Bibliographical note
Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/c1mb05202k
Source: dtu
Source-ID: n:oai:DTIC-ART:bl/323343906::29386
Publication: Research - peer-review › Journal article – Annual report year: 2011
Real-time detection of central carbon metabolism in living *Escherichia coli* and its response to perturbations

The direct tracking of cellular reactions in vivo has been facilitated with recent technologies that strongly enhance NMR signals in substrates of interest. This methodology can be used to assay intracellular reactions that occur within seconds to few minutes, as the NMR signal enhancement typically fades on this time scale. Here, we show that the enhancement of 13C nuclear spin polarization in deuterated glucose allows to directly follow the flux of glucose signal through rather extended reaction networks of central carbon metabolism in living *Escherichia coli*. Alterations in central carbon metabolism depending on the growth phase or upon chemical perturbations are visualized with minimal data processing by instantaneous observation of cellular reactions.

**General information**

State: Published  
Organisations: Carlsberg Laboratory, Albeda Research ApS  
Authors: Meier, S. (Intern), Jensen, P. R. (Intern), Duus, J. Ø. (Intern)  
Pages: 3133-3138  
Publication date: 2011  
Main Research Area: Technical/natural sciences

**Publication information**

Journal: FEBS Letters  
Volume: 585  
Issue number: 19  
ISSN (Print): 0014-5793  
Ratings:  
BFI (2018): BFI-level 1  
Web of Science (2018): Indexed yes  
BFI (2017): BFI-level 1  
Scopus rating (2017): CiteScore 3.43 SJR 1.991 SNIP 0.916  
Web of Science (2017): Indexed Yes  
BFI (2016): BFI-level 1  
Scopus rating (2016): CiteScore 3.48 SJR 1.967 SNIP 0.89  
Web of Science (2016): Indexed yes  
BFI (2015): BFI-level 1  
Scopus rating (2015): SJR 2.022 SNIP 0.923 CiteScore 3.49  
BFI (2014): BFI-level 1  
Scopus rating (2014): SJR 1.859 SNIP 0.87 CiteScore 3.19  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 1  
Scopus rating (2013): SJR 2.356 SNIP 0.982 CiteScore 3.71  
ISI indexed (2013): ISI indexed yes  
Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 1  
Scopus rating (2012): SJR 2.291 SNIP 0.913 CiteScore 3.67  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 1  
Scopus rating (2011): SJR 2.302 SNIP 0.833 CiteScore 3.5  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 1  
Scopus rating (2010): SJR 2.239 SNIP 0.792  
Web of Science (2010): Indexed yes  
BFI (2009): BFI-level 1  
Scopus rating (2009): SJR 2.17 SNIP 0.795  
Web of Science (2009): Indexed yes  
BFI (2008): BFI-level 1  
Scopus rating (2008): SJR 2.193 SNIP 0.786
Mr imaging agent or medium compressing hyperpolarised 13C alanine and methods of imaging wherein such an imaging medium is used.

The invention relates to hyperpolarised 13C-alanine, its use as imaging agent, an imaging medium comprising hyperpolarised 13C-alanine and methods of 13C-MR detection wherein such an imaging medium is used. Further, the invention relates to methods of producing hyperpolarised 13C-alanine.

General information
State: Published
Organisations: GE Healthcare
Authors: Gisselsson, Å. (Ekstern), Hansson, G. (Ekstern), Månsson, S. (Ekstern), In't Zandt, R. (Ekstern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Lerche, M. H. (Intern)
Publication date: 13 Oct 2010

Publication information
IPC: G01N 33/50 A I
Patent number: EP2237801
Date: 13/10/2010
Priority date: 03/02/2009
Priority number: WO2009EP51174
Original language: English
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: EP2237801
Publication: Research › Patent – Annual report year: 2010

Imaging of branched chain amino acid metabolism in tumors with hyperpolarized 13C ketoisocaproate
Powerful analytical tools are vital for characterizing the complex molecular changes underlying oncogenesis and cancer treatment. This is particularly true, if information is to be collected in vivo by noninvasive approaches. In the recent past, hyperpolarized 13C magnetic resonance (MR) spectroscopy has been employed to quickly collect detailed spectral information on the chemical fate of tracer molecules in different tissues at high sensitivity. Here, we report a preclinical study showing that α-ketoisocaproic acid (KIC) can be used to assess molecular signatures of tumors with hyperpolarized MR spectroscopy. KIC is metabolized to leucine by the enzyme branched chain amino acid transferase (BCAT), which is found upregulated in some tumors. BCAT is a putative marker for metastasis and a target of the proto-oncogene c-myc.
Very different fluxes through the BCAT-catalyzed reaction can be detected for murine lymphoma (EL4) and rat mammary adenocarcinoma (R3230AC) tumors in vivo. EL4 tumors show a more than 7-fold higher hyperpolarized $^{13}$C leucine signal relative to the surrounding healthy tissue. In R3230AC tumor on the other hand branched chain amino acid metabolism is not enhanced relative to surrounding tissues. The distinct molecular signatures of branched chain amino acid metabolism in EL4 and R3230AC tumors correlate well with ex vivo assays of BCAT activity.

General information
State: Published
Organisations: Imagnia AB, Carlsberg Laboratory
Authors: Karlsson, M. (Intern), Jensen, P. R. (Intern), in't Zandt, R. (Ekstern), Gisselsson, A. (Ekstern), Hansson, G. (Ekstern), Duus, J. Ø. (Intern), Meier, S. (Intern), Lerche, M. H. (Intern)
Pages: 729-736
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: International Journal of Cancer
Volume: 127
Issue number: 3
ISSN (Print): 0020-7136
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 2.182 SJR 3.152 CiteScore 6.4
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 5.93 SJR 2.991 SNIP 1.9
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.687 SNIP 1.547 CiteScore 4.94
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.603 SNIP 1.52 CiteScore 4.96
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 3.006 SNIP 1.903 CiteScore 5.93
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 2.854 SNIP 1.787 CiteScore 5.63
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.705 SNIP 1.576 CiteScore 5.07
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.579 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.37 SNIP 1.366
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.368 SNIP 1.34
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.325 SNIP 1.36
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.132 SNIP 1.378
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.068 SNIP 1.385
Study of molecular interactions with $^{13}$C DNP-NMR

NMR spectroscopy is an established, versatile technique for the detection of molecular interactions, even when these interactions are weak. Signal enhancement by several orders of magnitude through dynamic nuclear polarization alleviates several practical limitations of NMR-based interaction studies. This enhanced non-equilibrium polarization contributes sensitivity for the detection of molecular interactions in a single NMR transient. We show that direct $^{13}$C NMR ligand binding studies at natural isotopic abundance of $^{13}$C gets feasible in this way. Resultant screens are easy to interpret and can be performed at $^{13}$C concentrations below μM. In addition to such ligand-detected studies of molecular interaction, ligand binding can be assessed and quantified with enzymatic assays that employ hyperpolarized substrates at varying enzyme inhibitor concentrations. The physical labeling of nuclear spins by hyperpolarization thus provides the opportunity to devise fast novel in vitro experiments with low material requirement and without the need for synthetic modifications of target or ligands.

General information

State: Published
Organisations: Albeda Research ApS, Carlsberg Laboratory, GE Healthcare
Authors: Lerche, M. H. (Intern), Meier, S. (Intern), Jensen, P. R. (Intern), Baumann, H. (Ekstern), Petersen, B. O. (Ekstern), Karlsson, M. (Intern), Duus, J. Ø. (Intern), Ardenkjær-Larsen, J. H. (Intern)
Pages: 52-56
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information

Journal: Journal of Magnetic Resonance
Volume: 203
Issue number: 1
ISSN (Print): 1090-7807
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.963 SJR 1.182 CiteScore 2.57
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.37 SJR 1.016 SNIP 0.983
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.111 SNIP 1.07 CiteScore 2.88
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.113 SNIP 1.013 CiteScore 2.26
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.103 SNIP 0.937 CiteScore 2.41
ISI indexed (2013): ISI indexed yes
Method to produce hyperpolarised amino acids and aminosulphonic acids

The invention relates to a dynamic nuclear polarisation (DNP) method for producing hyperpolarised amino acids and amino sulphonic acids and compositions for use in the method.

General information
State: Published
Organisations: GE Healthcare
Authors: Lerche, M. H. (Intern), Karlsson, M. (Intern), Jensen, P. R. (Intern)
Publication date: 13 Aug 2009

Publication information
IPC: G01N 33/ 50 A I
Patent number: WO2009098191
Date: 13/08/2009
Priority date: 04/02/2008
Priority number: EP20080002001
Original language: English
Electronic versions:
WO2009098191A2_preparationaa.pdf

Bibliographical note
Mr imaging agent or medium compressing hyperpolarised 13C alanine and methods of imaging wherein such an imaging medium is used.

The invention relates to hyperpolarised 13C-alanine, its use as imaging agent, an imaging medium comprising hyperpolarised 13C-alanine and methods of 13C-MR detection wherein such an imaging medium is used. Further, the invention relates to methods of producing hyperpolarised 13C-alanine.

General information
State: Published
Organisations: GE Healthcare
Authors: Gisselsson, A. (Ekstern), Hansson, G. (Ekstern), Maansson, S. (Ekstern), Zandt, R. (Ekstern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Lerche, M. H. (Intern)
Publication date: 13 Aug 2009

Publication information
IPC: G01N 33/50 A1
Patent number: WO2009098192
Date: 13/08/2009
Priority date: 04/02/2008
Priority number: EP20080002002
Original language: English
Electronic versions:
WO2009098192A1_alanine.pdf

Bibliographical note
Also published as: US2011033390 (A1) JP2011511775 (A) EP2237801 (A1) CN101932341 (A)
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: WO2009098192
Publication: Research › Patent – Annual report year: 2009

Mr imaging agent, imaging medium and methods of imaging wherein such an imaging medium is used.

The invention relates to hyperpolarised 13C-a-ketoisocaproate, its use as imaging agent, an imaging medium comprising hyperpolarised 13C-a-ketoisocaproate and methods of 13C-MR detection wherein such an imaging medium is used. Further, the invention relates to methods of producing hyperpolarised 13C-a-ketoisocaproate.

General information
State: Published
Organisations: GE Healthcare
Authors: Gisselsson, A. (Ekstern), Hansson, G. (Ekstern), Maansson, S. (Ekstern), Zandt, R. (Ekstern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Lerche, M. H. (Intern)
Publication date: 2 Jul 2009

Publication information
IPC: A61K 49/20 A1
Patent number: WO2009080739
Date: 02/07/2009
Priority date: 21/12/2007
Priority number: NO20070006640
Original language: English
Electronic versions:
WO2009080739A1_KIC.pdf

Bibliographical note
Also published as: US2011038804 (A1) JP2011506015 (A) JP5351172 (B2) ES2379143 (T3) EP2222346 (A1) EP2222346 (B1) CN101951963 (A) CN101951963 (B) AT544474 (T)
Main Research Area: Technical/natural sciences
Source: espacenet
Composition and method for generating a metabolic profile using 13c-mr detection
The invention relates to a method of 13C-MR detection using an imaging medium comprising hyperpolarised 13C-fumarate and imaging media comprising hyperpolarised 13C-fumarate for use in said method.

General information
State: Published
Organisations: GE Healthcare
Authors: Gisselsson, A. (Ekstern), Hansson, G. (Ekstern), Maansson, S. (Ekstern), Zandt, R. (Ekstern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Lerche, M. H. (Intern)
Publication date: 25 Jun 2009

Imaging medium comprising hyperpolarised 13c-acetate and use thereof
The invention relates to a method of 13C-MR detection using an imaging medium comprising hyperpolarised 13C-acetate and to an imaging medium comprising hyperpolarised 13C-acetate.

General information
State: Published
Organisations: GE Healthcare
Authors: Gisselsson, A. (Ekstern), Hansson, G. (Ekstern), Maansson, S. (Ekstern), Zandt, R. (Ekstern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Lerche, M. H. (Intern)
Publication date: 5 Mar 2009

Imaging medium comprising hyperpolarised 13c-lactate and use thereof
The invention relates to a method of 13C-MR detection using an imaging medium comprising hyperpolarised 13C-lactate and to an imaging medium containing hyperpolarised 13C-lactate for use in said method.

General information
State: Published
Organisations: GE Healthcare
Authors: Gisselsson, A. (Ekstern), Hansson, G. (Ekstern), Maansson, S. (Ekstern), Zandt, R. (Ekstern), Karlsson, M. (Intern), Jensen, P. R. (Intern), Lerche, M. H. (Intern)
Publication date: 5 Mar 2009
Detection of low-populated reaction intermediates with hyperpolarized NMR

Hyperpolarized $^{13}$C NMR spectroscopy can provide the sensitivity and spectral resolution to detect, identify and quantify low-populated reaction intermediates, thus yielding direct chemical information on reaction mechanisms in real-time assays.

General information
State: Published
Organisations: Albeda Research ApS, Carlsberg Laboratory, GE Healthcare
Authors: Jensen, P. R. (Intern), Meier, S. (Intern), Ardenkjær-Larsen, J. H. (Intern), Duus, J. Ø. (Intern), Karlsson, M. (Intern), Lerche, M. H. (Intern)
Pages: 5168-5170
Publication date: 2009
Main Research Area: Technical/natural sciences
Publication information
Journal: Chemical Communications
Volume: 2009
Issue number: 34
ISSN (Print): 1359-7345
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 6.03 SJR 2.555 SNIP 1.127
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.06 SJR 2.538 SNIP 1.16
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.601 SNIP 1.295 CiteScore 6.7
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.692 SNIP 1.436 CiteScore 6.83
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.752 SNIP 1.372 CiteScore 6.73
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Hyperpolarized Amino Acids for In Vivo Assays of Transaminase Activity

General information
State: Published
Organisations: Imagnia AB, Carlsberg Laboratory
Authors: Jensen, P. R. (Intern), Karlsson, M. (Intern), Meier, S. (Intern), Duus, J. Ø. (Intern), Lerche, M. H. (Intern)
Pages: 10010-10012
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Chemistry: A European Journal
Volume: 15
Issue number: 39
ISSN (Print): 0947-6539
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
Production of hyperpolarized [1,4-C-13(2)]malate from [1,4-C-13(2)]fumarate is a marker of cell necrosis and treatment response in tumors

Dynamic nuclear polarization of C-13-labeled cell substrates has been shown to massively increase their sensitivity to detection in NMR experiments. The sensitivity gain is sufficiently large that if these polarized molecules are injected intravenously, their spatial distribution and subsequent conversion into other cell metabolites can be imaged. We have used this method to image the conversion of fumarate to malate in a murine lymphoma tumor in vivo after i.v. injection of hyperpolarized [1,4-C-13(2)] fumarate. In isolated lymphoma cells, the rate of labeled malate production was unaffected by coadministration of succinate, which competes with fumarate for transport into the cell. There was, however, a correlation with the percentage of cells that had lost plasma membrane integrity, suggesting that the production of labeled malate from fumarate is a sensitive marker of cellular necrosis. Twenty-four hours after treating implanted lymphoma tumors with etoposide, at which point there were significant levels of tumor cell necrosis, there was a 2.4-fold increase in hyperpolarized [1,4-C-13(2)] malate production compared with the untreated tumors. Therefore, the formation of hyperpolarized C-13-labeled malate from [1,4-C-13(2)] fumarate appears to be a sensitive marker of tumor cell death in vivo and could be used to detect the early response of tumors to treatment. Given that fumarate is an endogenous molecule, this technique has the potential to be used clinically.
Mechanistic details of mammalian metabolism in vivo and dynamic metabolic changes in intact organisms are difficult to monitor because of the lack of spatial, chemical, or temporal resolution when applying traditional analytical tools. These limitations can be addressed by sensitivity enhancement technology for fast in vivo NMR assays of enzymatic fluxes in tissues of interest. We apply this methodology to characterize organ-specific short chain fatty acid metabolism and the changes of carnitine and coenzyme A pools in ischemia reperfusion. This is achieved by assaying acetyl-CoA synthetase and acetyl-carnitine transferase catalyzed transformations in vivo. The fast and predominant flux of acetate and propionate signal into acyl-carnitine pools shows the efficient buffering of free CoA levels. Sizeable acetyl-carnitine formation from exogenous acetate is even found in liver, where acetyl-CoA synthetase and acetyl-carnitine transferase activities have been assumed sequestered in different compartments. In vivo assays of altered acetate metabolism were applied to characterize pathological changes of acetate metabolism upon ischemia. Coenzyme pools in ischemic skeletal muscle are

Tissue-specific Short Chain Fatty Acid Metabolism and Slow Metabolic Recovery after Ischemia from Hyperpolarized NMR in Vivo

Mechanistic details of mammalian metabolism in vivo and dynamic metabolic changes in intact organisms are difficult to monitor because of the lack of spatial, chemical, or temporal resolution when applying traditional analytical tools. These limitations can be addressed by sensitivity enhancement technology for fast in vivo NMR assays of enzymatic fluxes in tissues of interest. We apply this methodology to characterize organ-specific short chain fatty acid metabolism and the changes of carnitine and coenzyme A pools in ischemia reperfusion. This is achieved by assaying acetyl-CoA synthetase and acetyl-carnitine transferase catalyzed transformations in vivo. The fast and predominant flux of acetate and propionate signal into acyl-carnitine pools shows the efficient buffering of free CoA levels. Sizeable acetyl-carnitine formation from exogenous acetate is even found in liver, where acetyl-CoA synthetase and acetyl-carnitine transferase activities have been assumed sequestered in different compartments. In vivo assays of altered acetate metabolism were applied to characterize pathological changes of acetate metabolism upon ischemia. Coenzyme pools in ischemic skeletal muscle are
Reduced in vivo even 1 h after disturbing muscle perfusion. Impaired mitochondrial metabolism and slow restoration of free CoA are corroborated by assays employing fumarate to show persistently reduced tricarboxylic acid (TCA) cycle activity upon ischemia. In the same animal model, anaerobic metabolism of pyruvate and tissue perfusion normalize faster than mitochondrial bioenergetics.
Magnetic resonance imaging of pH in vivo using hyperpolarized 13C-labelled bicarbonate.

As alterations in tissue pH underlie many pathological processes, the capability to image tissue pH in the clinic could offer new ways of detecting disease and response to treatment. Dynamic nuclear polarization is an emerging technique for substantially increasing the sensitivity of magnetic resonance imaging experiments. Here we show that tissue pH can be imaged in vivo from the ratio of the signal intensities of hyperpolarized bicarbonate (H(13)CO(3)(-)) and (13)CO(2) following intravenous injection of hyperpolarized H(13)CO(3)(-). The technique was demonstrated in a mouse tumour model, which showed that the average tumour interstitial pH was significantly lower than the surrounding tissue. Given that bicarbonate is an endogenous molecule that can be infused in relatively high concentrations into patients, we propose that this technique could be used clinically to image pathological processes that are associated with alterations in tissue pH, such as cancer, ischaemia and inflammation.
Continuous molecular evolution of protein-domain structures by single amino acid changes

Protein structures cluster into families of folds that can result from extremely different amino acid sequences [11]. Because the enormous amount of genetic information generates a limited number of protein folds [2], a particular domain structure often assumes numerous functions. How new protein structures and new functions evolve under these limitations remains elusive. Molecular evolution may be driven by the ability of biomacromolecules to adopt multiple conformations as a bridge between different folds [3-6]. This could allow proteins to explore new structures and new tasks while part of the structural ensemble retains the initial conformation and function as a safeguard [7]. Here we show that a global structural switch can arise from single amino acid changes in cysteine-rich domains (CRD) of cnidarian nematocyst proteins. The ability of these CRDs to form two structures with different disulfide patterns from an identical cysteine pattern is distinctive [8]. By applying a structure-based mutagenesis approach, we demonstrate that a cysteine-rich domain can interconvert between two natively occurring domain structures via a bridge state containing both structures. Comparing cnidarian CRD sequences leads us to believe that the mutations we introduced to stabilize each structure reflect the birth of new protein folds in evolution.

General information
State: Published
Organisations: Joint Genome Institute, University of Basel, Ludwig-Maximilians-Universität, University of Heidelberg
Authors: Meier, S. (Intern), Jensen, P. R. (Intern), David, C. N. (Ekstern), Chapman, J. (Ekstern), Holstein, T. W. (Ekstern), Grzesiek, S. (Ekstern), Ozbek, S. (Ekstern)
Pages: 173-178
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Current Biology
Volume: 17
Issue number: 2
ISSN (Print): 0960-9822
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 5.26 SJR 4.296 SNIP 1.7
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.99 SJR 4.294 SNIP 1.533
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 4.705 SNIP 1.58 CiteScore 5.21
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 4.519 SNIP 1.59 CiteScore 5.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 4.896 SNIP 1.647 CiteScore 5.18
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 4.75 SNIP 1.77 CiteScore 4.98
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 4.848 SNIP 1.969 CiteScore 4.75
ISI indexed (2011): ISI indexed yes
Sequence-structure and structure-function analysis in cysteine-rich domains forming the ultrastable nematocyst wall

The nematocyst wall of cnidarians is a unique biomaterial that withstands extreme osmotic pressures, allowing an ultrafast discharge of the nematocyst capsules. Assembly of the highly robust nematocyst wall is achieved by covalent linkage of cysteine-rich domains (CRDs) from two main protein components, minicollagens and nematocyst outer wall antigen (NOWA). The bipolar minicollagens have different disulfide patterns and topologies in their N and C-terminal CRDs. The functional significance of this polarity has been elusive. Here, we show by NMR structural analysis that all representative cysteine-rich domains of NOWA are structurally related to N-terminal minicollagen domains. Natural sequence insertions in NOWA CRDs have very little effect on the tightly knit domain structures, nor do they preclude the efficient folding to a single native conformation. The different folds in NOWA CRDs and the atypical C-terminal minicollagen domain on the other hand can be directly related to different conformational preferences in the reduced states. Ultrastructural analysis in conjunction with aggregation studies argues for an association between the similar NOWA and N-terminal minicollagen domains in early stages of the nematocyst wall assembly, which is followed by the controlled association between the unusual structures of C-terminal minicollagen domains. (c) 2007 Elsevier Ltd. All rights reserved.
Nuclear magnetic resonance as a quantitative tool to study interactions in biomacromolecules.

High-resolution nuclear magnetic resonance (NMR) has emerged as one of the most versatile tools for the quantitative study of structure, kinetics, and thermodynamics of biomolecules and their interactions at atomic resolution. Traditionally, nuclear Overhauser enhancements (NOEs) and chemical shift perturbation methods are used to determine molecular geometries and to identify contact surfaces, but more recently, weak anisotropic orientation, anisotropic diffusion, and scalar couplings across hydrogen bonds provide additional information. Examples of such technologies are shown as applied to the quantitative characterization of function and thermodynamics of several biomacromolecules. In particular, (1) the structural and dynamical changes of the TipA multidrug resistance protein are followed upon antibiotic binding, (2) the trimer-monomer equilibrium and thermal unfolding of foldon, a small and very efficient trimerization domain of the 14 phagehead, is described in atomic detail, and (3) the changes of individual protein hydrogen bonds during thermal unfolding are quantitatively followed by scalar couplings across hydrogen bonds.
Improved detection of long-range residual dipolar couplings in weakly aligned samples by Lee-Goldburg decoupling of homonuclear dipolar truncation

Homonuclear H-1 residual dipolar couplings (RDCs) truncate the evolution of transverse H-1 magnetization of weakly aligned molecules in high-resolution NMR experiments. This leads to losses in sensitivity or resolution in experiments that require extended H-1 evolution times. Lee-Goldburg decoupling schemes have been shown to remove the effects of homonuclear dipolar couplings, while preserving chemical shift evolution in a number of solid-state NMR applications. Here, it is shown that the Lee-Goldburg sequence can be effectively incorporated into INEPT- or HMQC-type transfer schemes in liquid state weak alignment experiments in order to increase the efficiency of the magnetization transfer. The method is applied to the sensitive detection of (HN)-H-1-C-13 long-range RDCs in a three-dimensional HCN experiment. As compared to a conventional HCN experiment, an average sensitivity increase by a factor of 2.4 is obtained for a sample of weakly aligned protein G. This makes it possible to detect 170 long-range (HN-13C)-H-1 RDCs for distances up to 4.9 Ångstrom.

General information
State: Published
Organisations: University of Basel
Authors: Jensen, P. R. (Intern), Sass, H. (Ekstern), Grzesiek, S. (Ekstern)
Pages: 443-450
Publication date: 2004
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Biomolecular N M R
Volume: 30
Issue number: 4
ISSN (Print): 0925-2738
Ratings:
BFI (2018): BFI-level 1
Improvement of hydrogen bond geometry in protein NMR structures by residual dipolar couplings - an assessment of the interrelation of NMR restraints

We have examined how the hydrogen bond geometry in three different proteins is affected when structural restraints based on measurements of residual dipolar couplings are included in the structure calculations. The study shows, that including restraints based solely on (H-N)-H-1-N-15 residual dipolar couplings has pronounced impact on the backbone rmsd and Ramachandran plot but does not improve the hydrogen bond geometry. In the case of chymotrypsin inhibitor 2 the addition of (C-O)-C-13-C-13(alpha) and N-15-(C-O)-C-13 one bond dipolar couplings as restraints in the structure calculations improved the hydrogen bond geometry to a quality comparable to that obtained in the 1.8 Angstrom resolution X-ray structure of this protein. A systematic restraint study was performed, in which four types of restraints, residual dipolar couplings, hydrogen bonds, TALOS angles and NOEs, were allowed in two states. This study revealed the importance of using several types of residual dipolar couplings to get good hydrogen bond geometry. The study also showed that using a small set of NOEs derived only from the amide protons, together with a full set of residual dipolar couplings resulted in structures of very high quality. When reducing the NOE set, it is mainly the side-chain to side-chain NOEs that are removed. Despite of this the effect on the side-chain packing is very small when a reduced NOE set is used, which implies that the over all fold of a protein structure is mainly determined by correct folding of the backbone.

General information
State: Published
Organisations: University of Copenhagen
Authors: Jensen, P. R. (Intern), Axelsen, J. B. (Ekstern), Lerche, M. H. (Intern), Poulsen, F. M. (Ekstern)
Pages: 31-41
Publication date: 2004
Main Research Area: Technical/natural sciences

Publication Information
Journal: Journal of Biomolecular N M R
Volume: 28
Issue number: 1
ISSN (Print): 0925-2738
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.699 SJR 1.371 CiteScore 2.45
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.891 SNIP 0.884 CiteScore 2.52
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.027 SNIP 1.157 CiteScore 3.72
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.153 SNIP 0.894 CiteScore 2.96
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.806 SNIP 1.02 CiteScore 3.51
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 2.429 SNIP 1.252 CiteScore 3.75
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.341 SNIP 1.224 CiteScore 3.75
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.969 SNIP 0.918
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.902 SNIP 0.784
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.692 SNIP 0.651
Scopus rating (2007): SJR 1.811 SNIP 0.838
Scopus rating (2006): SJR 2.096 SNIP 0.948
High-Accuracy Residual $^1$H\textsuperscript{-}13C and $^1$H\textsuperscript{-}$^1$H Dipolar Couplings in Perdeuterated Proteins

Truncation by the presence of many short-range residual dipolar couplings (RDCs) hinders the observation of long-range RDCs in weakly aligned biomacromolecules. Perdeuteration of proteins followed by reprotonation of labile hydrogen positions greatly alleviates this problem. Here we show that for small perdeuterated proteins, a large number (up to 10 in protein G) of long-range RDCs to $^{13}$C and $^1$HN can be observed from individual amide protons. The $^1$HN $\leftrightarrow$ 13C RDCs comprise correlations to $^{13}$C\textalpha{}, $^{13}$C\textbeta{}, and $^{13}$C\textsuperscript{'} nuclei of the same and the preceding amino acid, as well as $^{13}$C\textsuperscript{'} nuclei of hydrogen-bonded amino acids. The accuracy of the coupling constants is very high and defines individual internuclear distances to within few picometers. Deviations between measured RDC values and values predicted from the 1.1 Å crystal structure of protein G are mainly found in two surface-exposed loop regions. The deviations show a strong correlation to the B-factor of the crystal structure. Copyright © 2003 American Chemical Society.
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 13.18 SJR 7.492 SNIP 2.596
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 6.775 SNIP 2.63 CiteScore 12.81
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 6.294 SNIP 2.587 CiteScore 11.92
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 5.993 SNIP 2.466 CiteScore 11.38
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 6.211 SNIP 2.38 CiteScore 10.37
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 5.478 SNIP 2.321 CiteScore 9.94
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 5.167 SNIP 2.138
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 5.06 SNIP 2.16
Web of Science (2008): Indexed yes
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 4.662 SNIP 2.252
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 4.413 SNIP 2.223
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 3.841 SNIP 2.203
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 3.421 SNIP 2.236
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 3.223 SNIP 2.345
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 3.506 SNIP 2.15
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 3.972 SNIP 2.163
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 3.438 SNIP 2.133

Original language: English

amino acid, carbon, hydrogen, protein, proton, article, calculation, chemical structure, crystal, crystal structure, dynamics, geometry, hydrogen bond, macromolecule, molecule, nuclear magnetic resonance, parameter, Amides, Carbon Isotopes, Deuterium, Nuclear Magnetic Resonance, Biomolecular, Proteins, CHEMISTRY, 2D

DOIs:
10.1021/ja028740q
Synthesis and structural properties of 5,17-bis (N-methyl-N-arylaminocarbonyl)calix[4]arenes. Directing the substituents toward the cavity by use of the cis-generating property of the N-methylaminocarbonyl linker

A series of cone 5,17-bis(N-arylaminocarbonyl)calix[4]arenes were synthesized and N-methylated using an easy and high-yielding methylation procedure. The structures of the cone 5,17-bis(Nmethyl-N-arylaminocarbonyl)calix[4]arenes were studied in solution by NMR spectroscopy and in the solid state by X-ray structural resolution. The use of the N-methylaminocarbonyl linker between the calix[4]arene and the aromatic substituent was found to have a dominant influence on the molecular structure, forcing the substituent toward the cavity of the calix[4]arene regardless of the size of the substituent. The linker may be a very useful structure generator when considering the design of molecular receptors.

General information
State: Published
Organisations: Solar Energy Programme, Risø National Laboratory for Sustainable Energy, Department of Energy Conversion and Storage, Functional organic materials, Department of Chemistry, University of Copenhagen
Authors: Krebs, F. C. (Intern), Larsen, M. (Intern), Jørgensen, M. (Intern), Jensen, P. R. (Intern), Bielecki, M. (Ekstern), Schaumburg, K. (Ekstern)
Pages: 9872-9879
Publication date: 1998
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Organic Chemistry
Volume: 63
ISSN (Print): 0022-3263
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): SNIP 0.997 SJR 1.846 CiteScore 4.55
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.59 SJR 2.001 SNIP 1.035
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.997 SNIP 1.166 CiteScore 4.69
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.007 SNIP 1.219 CiteScore 4.69
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.092 SNIP 1.169 CiteScore 4.51
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.286 SNIP 1.223 CiteScore 4.31
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.265 SNIP 1.239 CiteScore 4.43
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 2.127 SNIP 1.169
BFI (2009): BFI-level 2