Agència de Gestió d'Ajuts Universitaris i de Recerca
AGAUR

Memòria justificativa de recerca de les convocatòries BCC, BE, BP, CTP-AIRE, INEFC i PIV

La memòria justificativa consta de les dues parts que venen a continuació:
1.- Dades bàsiques i resums
2.- Memòria del treball (informe científic)

Tots els camps són obligatoris

1.- Dades bàsiques i resums

Nom de la convocatòria

BP

Llegenda per a les convocatòries:

BCC	Convocatòria de beques per a joves membres de comunitats catalanes a l'exterior
BE	Beques per a estades per a la recerca fora de Catalunya
BP	Convocatòria d'ajuts postdoctorals dins del programa Beatriu de Pinós
CTP-AIRE	Ajuts per accions de cooperació en el marc de la comunitat de treball dels Pirineus. Ajuts de mobilitat de personal investigador.
INEFC	Beques predoctorals i de col·laboració, dins de l'àmbit de l'educació física i l'esport i les ciències aplicades a l'esport
PIV	Beques de recerca per a professors i investigadors visitants a Catalunya

Títol del projecte: ha de sintetitzar la temàtica científica del vostre document.
New Radicals and new applications of Dynamic Nuclear Polarization of Biological Systems

Dades de l'investigador o beneficiari

Nom: Ildefonso
Cognoms: Marín Montesinos

Correu electrònic
ildefonso.marin@irbbarcelona.org

Dades del centre d'origen

Institut de Recerca Biomedica (IRB-Barcelona)

Número d'expedient
2009 BP-B00244

Paraules clau: cal que esmenteu cinc conceptes que defineixin el contingut de la vostra memòria.
Dynamica Nuclear Polarization, Nuclear Magnetic Resonance, Hyperpolarization, quadrupolar nuclei, radical

Data de presentació de la justificació
8/01/2013

Nom i cognoms i signatura del/de la investigador/a	Vist i plau del/de la responsable de la sol·licitud

Resum del projecte: cal adjuntar dos resums del document, l'un en anglès i l'altre en la llengua del document, on s'esmenti la durada de l'acció

Resum en la llengua del projecte (màxim 300 paraules)

Dynamic Nuclear Polarization (DNP) is an emerging technique that could revolutionize the NMR study of small molecules at very low concentrations by the increase in sensitivity that results from transfer of polarization between electronic and nuclear spins. Although the underlying physics has been known for a long time, in the last few years there has been a lot of excitement on the chemistry and biology NMR community caused by the demonstration that the highly polarized nuclei that are prepared in solid state at very low temperatures (1-2 K) could be rapidly transferred to liquid samples at room temperature and studied in solution by conventional NMR techniques. In favorable cases several order of magnitude increases in sensitivity have been achieved. The technique is now mature enough that a commercial instrument is available.
The efficiency of DNP depends on two crucial aspects: i) the efficiency of the nuclear polarization process and ii) the efficiency of the transfer from the initial solid state to the fluid state in which NMR is measured. The preferred areas of application (iii) will be dictated by situations in which the low concentration of the sample or its intrinsic low receptivity are the limiting factors .

(i) Exploring new radicals for DNP.

The efficiency of nuclear polarization is strongly dependent on the type of radicals used and the relative distance between the radical site and the position of the nuclei of interest. One of the objectives of the project will be the exploration of different radicals including some with a new design, which will be produced in collaboration with synthetic groups in the IRB and the ICMAB.These new radical will exploit the linkage between a supramolecular receptor and a stable organic radical. The concept is that the receptor will reversibly bind the species to be polarized and reduce the average distance to the radical, enhancing the polarization efficiency.

(ii) Increasing the efficiency of the transfer process.

The highly nuclear polarized species are transferred to the NMR instrument by quickly melting-dissolving the sample in hot solvent and transferring the solution to the NMR in few seconds. During this time, fast relaxing species will lose a substantial amount of polarization, decreasing the efficiency of the process. We believe that there is room for improvement by exploring the use of a flow probe, that requires a much smaller volumes of solution and could reduce the waiting time for the decay of fluid turbulence that destroy the sample homogeneity. The possibility of immobilizing the radicals, achieving an effective separation of the radical and the analyte would also result in an increase in sensitivity as the effective relaxation time of the molecules of interest would be increased.

(iii) Applications:

(a) DNP of gases. The polarization of gases like129Xe. Other gas samples like CO2 could also be polarized and would open a number of applications in the biological field. Gas samples are intrinsically of very low concentration as compared to condensed phases. The use of a versatile hyperpolarization method would open an entire field of research.

(b) Polarization of Lithium ion. Lithium ions in solution have also very long relaxation times and have very similar ionic radii to Magnesium, a very common biologically relevant ion. By using proper receptor-radicals (see point i) the use of DNP-hyperpolarized lithium may become a useful tool in many biologically and chemically relevant systems.

(c) Drug screening. The increase in sensitivity obtained by DNP creates a non-equilibrium state that could be used in the context of drug screening (analogous to the widely used STD experiment) by exploiting the different correlation times of free and bound species. The possibility of studying low concentration samples may allow the use of chemical shift changes more efficiently for ligands with moderate affinity.

(d) Metabolomics. The enhanced sensitivity provided by the DNP method may provide a new view of the low concentration metabolites in biological samples.

Resum en anglès (màxim 300 paraules)

Dynamic Nuclear Polarization (DNP) is an emerging technique that could revolutionize the NMR study of small molecules at very low concentrations by the increase in sensitivity that results from transfer of polarization between electronic and nuclear spins. Although the underlying physics has been known for a long time, in the last few years there has been a lot of excitement on the chemistry and biology NMR community caused by the demonstration that the highly polarized nuclei that are prepared in solid state at very low temperatures (1-2 K) could be rapidly transferred to liquid samples at room temperature and studied in solution by conventional NMR techniques. In favorable cases several order of magnitude increases in sensitivity have been achieved. The technique is now mature enough that a commercial instrument is available.
The efficiency of DNP depends on two crucial aspects: i) the efficiency of the nuclear polarization process and ii) the efficiency of the transfer from the initial solid state to the fluid state in which NMR is measured. The preferred areas of application (iii) will be dictated by situations in which the low concentration of the sample or its intrinsic low receptivity are the limiting factors .

(iv) Exploring new radicals for DNP.

The efficiency of nuclear polarization is strongly dependent on the type of radicals used and the relative distance between the radical site and the position of the nuclei of interest. One of the objectives of the project will be the exploration of different radicals including some with a new design, which will be produced in collaboration with synthetic groups in the IRB and the ICMAB.These new radical will exploit the linkage between a supramolecular receptor and a stable organic radical. The concept is that the receptor will reversibly bind the species to be polarized and reduce the average distance to the radical, enhancing the polarization efficiency.

(v) Increasing the efficiency of the transfer process.

The highly nuclear polarized species are transferred to the NMR instrument by quickly melting-dissolving the sample in hot solvent and transferring the solution to the NMR in few seconds. During this time, fast relaxing species will lose a substantial amount of polarization, decreasing the efficiency of the process. We believe that there is room for improvement by exploring the use of a flow probe, that requires a much smaller volumes of solution and could reduce the waiting time for the decay of fluid turbulence that destroy the sample homogeneity. The possibility of immobilizing the radicals, achieving an effective separation of the radical and the analyte would also result in an increase in sensitivity as the effective relaxation time of the molecules of interest would be increased.

(vi) Applications:

(a) DNP of gases. The polarization of gases like129Xe. Other gas samples like CO2 could also be polarized and would open a number of applications in the biological field. Gas samples are intrinsically of very low concentration as compared to condensed phases. The use of a versatile hyperpolarization method would open an entire field of research.

(b) Polarization of Lithium ion. Lithium ions in solution have also very long relaxation times and have very similar ionic radii to Magnesium, a very common biologically relevant ion. By using proper receptor-radicals (see point i) the use of DNP-hyperpolarized lithium may become a useful tool in many biologically and chemically relevant systems.

(c) Drug screening. The increase in sensitivity obtained by DNP creates a non-equilibrium state that could be used in the context of drug screening (analogous to the widely used STD experiment) by exploiting the different correlation times of free and bound species. The possibility of studying low concentration samples may allow the use of chemical shift changes more efficiently for ligands with moderate affinity.

(d) Metabolomics. The enhanced sensitivity provided by the DNP method may provide a new view of the low concentration metabolites in biological samples.

2.- Memòria del treball (informe científic sense limitació de paraules). Pot incloure altres fitxers de qualsevol mena, no més grans de 10 MB cadascun d'ells.

In the framework of the "New Radicals and applications for Dynamic Nuclear Polarization", in the six month in which the project had been working, the line: hyperpolarization of nitrogen-14 by Dynamic Nuclear Polarization, has been put in place.
DNP All measurements made in the following studies have been conducted on the instrument Oxford HyperSense Dissolution Instrument for DNP [1].

Hyperpolarization of nitrogen-14.

DNP has become an important tool to increase the sensitivity of the NMR experiments, both solid and liquid samples [2], [3]. New applications have appeared in recent years in different fields in physics, chemistry, biology and medicine. Therefore, DNP is expected to become an essential addition to the NMR technique [4], [5], [6], [7]. However, applications are restricted to a small number of nuclei, mainly with spin ½ (^{1}H, ^{13}C and ^{15}N). Recently, the technique has been applied to the nucleus ^{89}Y with low gyromagnetic radius [8] and ^{6}Li, quadrupolar nucleus, which due to its low quadrupolar constant may be considered as spin ½ [9]. Therefore, it is necessary to expand the DNP technique to quadrupolar nuclei and low gyromagnetic radius (majority in the periodic table) which are difficult to detect using conventional NMR.
^{14}N is a good example of quadrupolar nucleus gyromagnetic radius and low, difficult to detect by NMR with good sensitivity. ^{14}N has spin 1, 99.6% natural abundance and a relatively large quadrupole moment (Q ~ 2 fm2). The DNP hyperpolarization could make it easier to observation by NMR, opening up new applications.
Most compounds have T_1 (longitudinal relaxation time) very small, but if the environment around the ^{14}N nucleus is spherical symmetrically, T_1 can be enough to be of use DNP. The molecules used in these studies were tetramethylammonium chloride (TMA), choline (Cho), phosphocholine (PCho) and glicerolfosfocolina (GPcho).

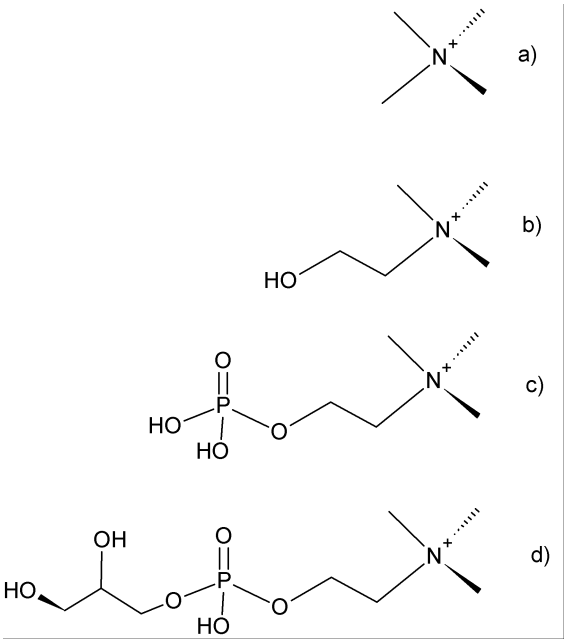

Figure.1. a) tetramethylammonium, b) Choline, c) and d Phosphocholine) Glycerofosfocolina

TMA molecule was chosen as standard for the optimization of the different variables for DNP hyperpolarization. The radical used is ox63 (trityl) at a concentration of 15mM and the matrix was a mixture of 50% by volume of deuterated water / glycerol.

The microwave frequency sweep (GHz-94 230 94 080 GHz) shows four polarization bands, two positive and two negative (figura.2.a)). The resonance signal of hyperpolarized ^{14}N is acquired ex situ, after dissolution and transfer to a 500MHz NMR spectrometer. This result does not follow the expected pattern of low gyromagnetic ratio nuclei. The figure.2.b) shows the evolution of the magnetization during the DNP process at 94,095 GHz and 94,135 GHz and it shows a totally different behavior than expected, with a small initial growth and subsequent substantial jump in polarization. Similar effects were observed in choline hyperpolarization. Several explanations have been proposed and we are currently working in them.

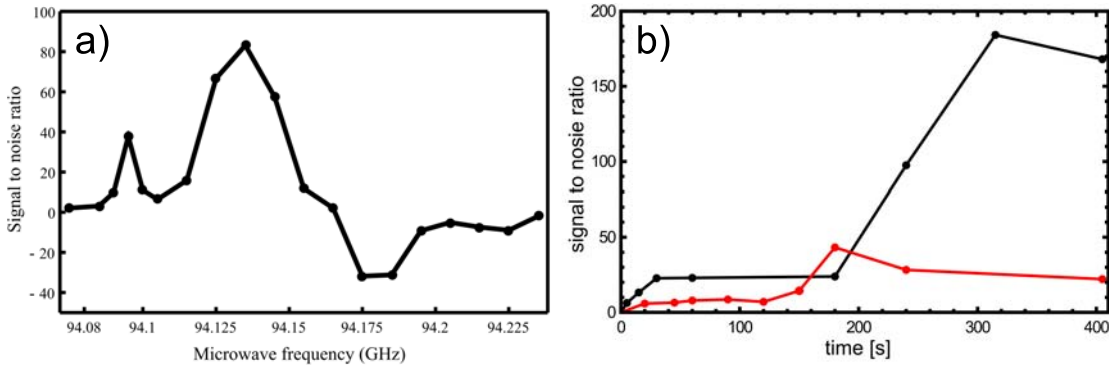

Figure.2. a) Microwave sweep for a sample of TMA with Ox63. b) Polarization buildup defor a sample of TMA with Ox63 at frequencies of 94.135 GHz (black) and 94.095 GHz (red).

The main results obtained in this part of the project are:
1. It has been possible to hyperpolarize nitrogen-14 molecules with certain high performance spherical symmetry. These molecules are compounds of biological interest such as choline, phosphocholine or glicerolfosfocolina.
2. Signal increases are substantial, yielding approximately a factor of 2500 to 600 tetramethylammonium for hill
3. The DNP mechanism appears to follow the pattern of other nuclei of low gyromagnetic radius, yielding several bands of polarization in the microwave sweeps profiles. Furthermore, the growth curves of polarization do not follow an exponential growth.

References.

1. A. Sowerby, *Chem. Ind.*, **17**, 21 (2005).
2. U.Günther, *Topics in Current Chemistry*, (2011).
3. R. Griffin et al., *J. Chem. Phys.*, **128**, 052211 (2008).
4. L. Frydman et al, *Nature Physics*, **3**, 415 (2007) .
5. L.Emsley et al, *J. Am. Chem. Soc.*, **132**, 15459 (2010).
6. Goldman et al, *Proc. Natl. Acad. Sci., USA*, **103**, 11270 (2006).
7. J. H. Ardenkjaer-Larsen et al., *NMR Biomed.*, **24**, 927 (2011).
8. L.Lumata et al., *J. Am. Chem. Soc.*, **133**, 8673 (2011).
9. R. Gruetter et al., *Magn. Reson. Med.*, **61**, 1489 (2009).