

Advanced Solutions for the Investigation of Challenging Biological Systems by NMR Spectroscopy



Global Overview - Who are we?



- NMR-Bio arose from the collaboration between the “Institut de Biologie Structurale” (IBS) and the Laboratory of Metal Chemistry and Biology (BIG/LCBM), in Grenoble, France.
- NMR-Bio, hosted by IBS, manufactures and distributes patented precursors for the production of methyl-specific labelled proteins with stable isotopes.
- NMR-Bio offers a wide range of customized NMR services:
 - Precursors for selective isotopic labelling.
 - customized synthesis of isotopically labelled protein.
 - High-field NMR data acquisition and analysis.
- Our partners:

NMR-Bio is located in the heart of one of the most vibrant European hubs for structural biology



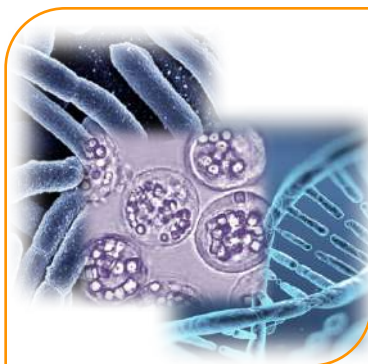
- Our patents:
 - WO/2011/083356
 - WO/2014/024151A1
 - WO/2017/055056

Global approach- A new perspective for drug development and target validation



Precursors for selective isotopic labeling

- *The* latest generation of NMR optimised precursors for specific (^{13}C , ^1H)-labelling of methyl groups in deuterated proteins.
- The precursors are supplied as user-friendly kits, ready for direct use in culture medium without further modification.
- Kits are packaged in vials precalibrated for 1L *E. coli* perdeuterated culture medium.



Custom Protein Production

- **Expression systems: bacteria, cell-free, insects cells, HEK293, CHOs**
- *In vivo* production of U- ^{2}H , ^{13}C , ^{15}N proteins;
- *In vivo* production of U- ^{2}H , $^{13}\text{CH}_3$ large proteins;
- *In vivo* production of mutant libraries for $^{13}\text{CH}_3$ NMR spectra assignment;
- *In vitro* production of labeled proteins offering a total ^{15}NH back-protonation avoiding any critical refolding;
- *In vitro* production of challenging U- ^{2}H , $^{13}\text{CH}_3$ proteins (e.g. membrane proteins) using our cost-effective technology;



Structural studies of supra-molecular proteins (NMR & EM)

- High field NMR data acquisition (600-950 MHz);
 - NMR spectra assignment;
 - Protein quality control;
 - Structure & dynamic studies of proteins;
 - Drug screening and mapping interactions of protein targets;
 - Characterisation of protein-drug and protein-protein interactions
- Negative stain EM quality control of proteins;
 - Cryo-EM Structural study of large biological assemblies.

Precursors for selective isotopic labelling

Custom Protein Production

Structural studies of proteins



Methyl-specific isotope-labelling techniques paved the way for the characterization of supra-molecular biological systems and protein complexes by NMR spectroscopy.

NMR-Bio user-friendly kits for $^{13}\text{CH}_3$ and $^{13}\text{CHD}_2$ incorporation, with **regio- and stereospecificity**, allow an increase in sensitivity and resolution of NMR spectra. In our ready-to-use product pipeline we propose:

- NMR-Bio Rich Culture Media: For the production of [^1H , ^{13}C]-Methyl, U-[D] proteins, increasing protein yield by up to 3-fold.
- NMR-BIO Combinatorial Labelling Kits: For the simultaneous labelling of any combination of methyl-containing residue including Ala, Ile, Leu, Met, Thr & Val methyl groups.
- NMR-BIO Kits for large protein labelling: For investigation of protein assemblies with molecular weight up to 1 MDa.
- NMR-BIO Kits for solid-state & dynamic NMR studies.



NMR-Bio labelling technology allows NMR monitoring of protein in its physiological state for interaction mapping, target and drug validation and drug discovery

E. Coli

- ^2H , ^{13}C , ^{15}N uniform labelling
- $^{13}\text{CH}_3$ specific labelling of large U-[D]proteins
- **Production of mutant libraries for assignment**
- Production of challenging proteins
- Rich culture media for specific labelling
- ...etc



Cell-free using *E. coli* extract cell

- Soluble and membrane proteins
- Disulfide-bridged proteins (ex: FABs, mAbs...)
- Co-expression, large assemblies
- ^2H , ^{13}C , ^{15}N Isotopic labelling
- ^{15}NH back-protonation avoiding any critical refolding
- Large scale production > 1 mg
- ...etc



Insect cells

- Insect cell baculovirus system
- Intracellular or secreted proteins
- ^2H , ^{13}C , ^{15}N uniform labelling
- $^{13}\text{CH}_3$ specific labelling of large U-[D]proteins
- Production of challenging proteins (kinases, viral proteins...)
- ...etc



Mammalian cells

- HEK293 cells, CHOs
- ^2H , ^{13}C , ^{15}N uniform labelling
- $^{13}\text{CH}_3$ specific labelling of large U-[D]proteins
- Production of proteins of pharmaceutical interest (ex: mAbs)
- ...etc



50-µm

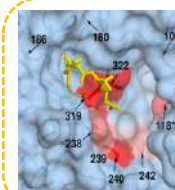
Precursors for selective isotopic labelling

Custom Protein Production

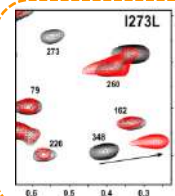
Structural studies of proteins



NMR-BIO Value



Drug screening and mapping interactions of protein targets



Characterisation of protein-drug and protein-protein interactions



Structure & dynamic studies of enzymes

Our tools

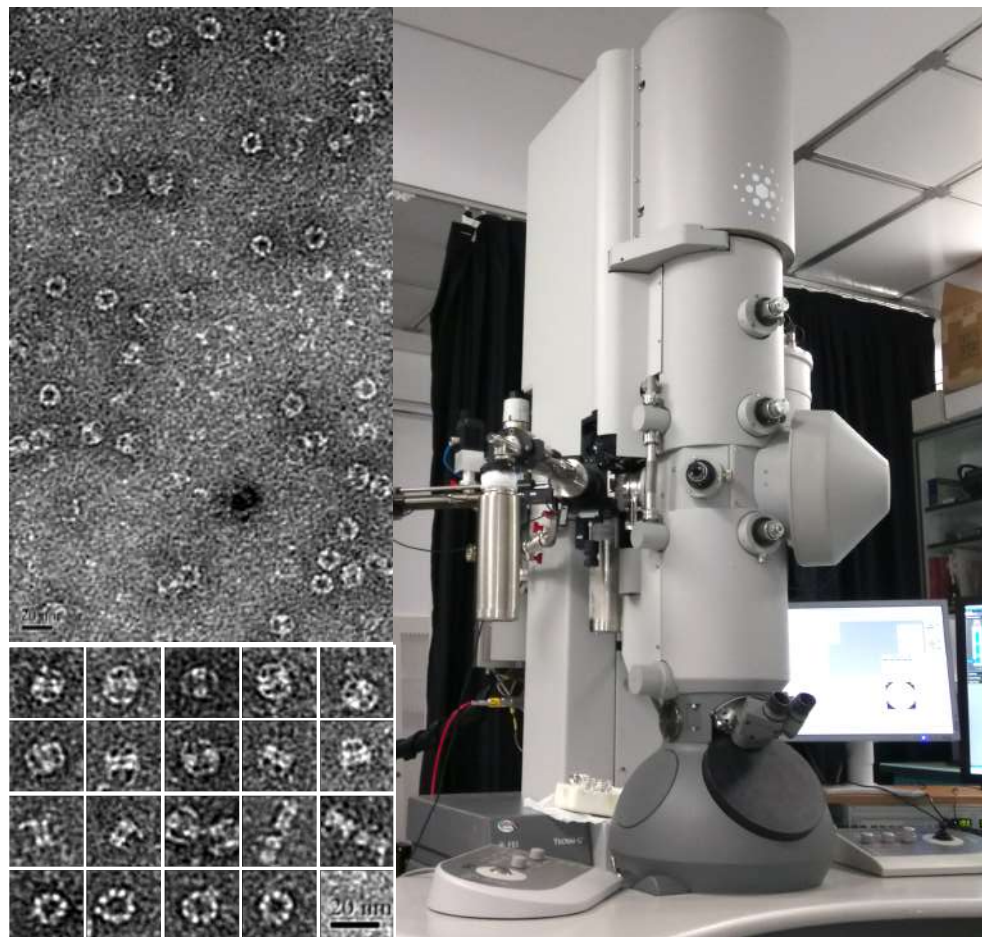
- Sophisticated detection probes for solid and solution state NMR spectroscopy;
- ^1H , ^2H , ^{13}C , ^{15}N , ^{19}F & ^{31}P NMR;
- State-of-the-art NMR data acquisition;
- Small quantities of sample (<1 nmol of protein).

An in depth NMR study of Structure, Dynamics & interactions of supra-molecular proteins. Custom High filed NMR data acquisition and analysis

Precursors for selective isotopic labelling

Custom Protein Production

Structural studies of proteins



NMR-BIO Value

- Negative stain EM quality control of proteins
- Cryo-EM Structural study of large biological assemblies.

Our tools (microscopes)

For Cryo EM

- FEI Tecnai POLARA 300 kV FEG equipped with K2 direct electron detector
- FEI Tecnai F20 200 kV FEG

For NS EM

- T12 120 kV LaB6
- F20 200 kV FEG

A service in collaboration with IBS scientists

Examples of our publications

Angewandte
International Edition
Chemie



A Journal of the
Gesellschaft
Deutscher Chemiker

Communication Full Access

Stereospecific Isotopic Labeling of Methyl Groups for NMR Spectroscopic Studies of High-Molecular-Weight Proteins*

Pierre Gans Dr., Olivier Hamelin Dr., Remy Sounier Dr., Isabel Ayala, M. Asunción Durá Dr., Carlos D. Amero Dr., Marjolaine Noirclerc-Savoie Dr., Bruno Franzetti Dr., ... See all authors



Structural Genomics pp 229-244 | [Cite as](#)

A Cost-Effective Protocol for the Parallel Production of Libraries of $^{13}\text{CH}_3$ -Specifically Labeled Mutants for NMR Studies of High Molecular Weight Proteins

Authors [Authors and affiliations](#)

Elodie Crublet, Rime Kerfah, Guillaume Mas, Marjolaine Noirclerc-Savoie, Violaine Lantez, Thierry Vernet, Jerome Boisbouvier

SCIENCE ADVANCES | RESEARCH ARTICLE

STRUCTURAL BIOLOGY

Unraveling self-assembly pathways of the 468-kDa proteolytic machine TET2

Pavel Macek,^{1,2,3} Rime Kerfah,^{1,2,3*} Elisabetta Boeri Erba,^{1,2,3} Elodie Crublet,^{1,2,3*} Christine Moriscot,^{1,2,3} Guy Schoehn,^{1,2,3} Carlos Amero,^{4†} Jerome Boisbouvier^{1,2,3†}

J Biomol NMR (2015) 63:389–402
DOI 10.1007/s10858-015-9998-4



ARTICLE

CH_3 -specific NMR assignment of alanine, isoleucine, leucine and valine methyl groups in high molecular weight proteins using a single sample

Rime Kerfah^{1,2,3} · Olivier Hamelin⁴ · Jérôme Boisbouvier^{1,2,3} · Dominique Marion^{1,2,3,5}



Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
Structural Biology

Methyl-specific isotopic labeling: a molecular tool box for solution NMR studies of large proteins

Rime Kerfah^{1,2,3,6}, Michael J Plevin⁴, Remy Sounier⁵, Pierre Gans^{1,2,3} and Jerome Boisbouvier^{1,2,3}



ChemComm

View Article Online
DOI: 10.1039/C6CC04484K

COMMUNICATION

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www.rsc.org/

Sensitive proton-detected solid-state NMR spectroscopy of large proteins with selective CH_3 labelling: application to the 50S ribosome subunit

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