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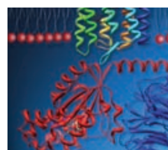

Solid-state NMR on Larger Biomolecules

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Introduction

In the last years, remarkable progress has been made to probe molecular structure of biological systems using Magic Angle Spinning solid-state NMR (ssNMR). Prominent examples relate to research areas that have remained challenging to classical structural biology methods such as membrane proteins[1,2] and protein fibrils (see, e.g., Ref [3,4,5]). In addition, ssNMR continues to contribute to a structural understanding of basic biological processes including enzyme catalysis or photosynthesis and is capable of studying far more complicated heterogeneous biomolecular systems such as bacterial cell walls[6] or inclusion bodies[7,8]. Clearly, these advancements would have been impossible without methodological and instrumental progress in the field of ssNMR and the pioneering work of Griffin, Opella, Cross, Torchia and others in the field of biomolecular ssNMR. Yet, a decade ago, it was still unclear whether one would be able to obtain sequential assignments of larger proteins, not to mention the determination of their 3D structures from ssNMR data. Since then, ssNMR progress has been substantial and improvements in the field of solution-state NMR continue to cross fertilize and speed up developments in solid-state NMR. Finally, the revolutionary developments in biochemistry and molecular biology in combination with isotope-labelling, and in more general sense, the ability to design biomolecular sample preparations for ssNMR studies has played a critical role. With further increasing molecular size, for example relating to proteins comprising several hundred amino acids, new challenges and opportunities lay ahead of us.

Biomolecular (Supra)structure & Dynamics

Isotope-labelling plays a critical role in establishing structural constraints using CC, CHHC or related correlation methods in a biomolecular context. Such experiments have thus far been crucial to determine molecular structures of larger peptides and proteins from MAS ssNMR data (for reviews, see e.g. Ref.[9,10]). Usually, uniform (^{13}C,^{15}N) isotope labeling is employed to perform an initial spectroscopic characterization of the biomolecule of interest. In polypeptides, a simple comparison of the 2D (^{13}C,^{13}C) cross peak pattern can be sufficient to assess structural homogeneity and short-range order. In the next stage, ^{15}N spectra and, in particular, (^{15}N,^{13}C) 2D data further report on molecular order and ^{1}H bonding. In such correlation experiments, polarization transfer can either involve through-space and through-bond interactions. The choice which polarization transfer scheme is most suitable may depend on experimental parameters such as available MAS rate, sample conditions (for example proteoliposomes vs. microcrystals) and intrinsic molecular properties such as mobility and polymorphism.

Using uniformly labeled samples, near-complete resonance assignments of several proteins encompassing about 100 amino-acids have been reported. In larger systems, three and potentially higher-dimensional correlation experiments that have already been described in the literature (see, e.g., Ref.[11,12]) are needed. Moreover, alternative isotope-labelling strategies play a prominent role to reduce spectral crowding in larger systems. For a long time, (see e.g. Ref.[13]) "forward" labeling where isotope-labeled amino acids are added to the growth medium have been used. Although such methods often do not totally remove spectral ambiguity, they strongly reduce spectroscopic overlap. "Pair-wise" amino acid labeling may be sufficient to isolate ssNMR signals of a specific residue. Recent applications of such strategies for example relate to larger membrane proteins[14,15]. In addition, block labeling[16,17] as well as reverse[18] labeling strategies have successfully been used in ssNMR. In these experiments, a dedicated set of amino-acid precursors or amino acids is used during expression. The combination of such measures was, for example, employed in the case of microcrystalline proteins[19], amyloid[4] and membrane proteins[20,21]. With increasing molecular size another option can be segmental labeling, in which only a fraction of the protein is studied and data are compared to larger constructs. Such "divide-and-conquer" strategies were for example employed to reassembled proteins[22] and multi-domain membrane proteins[23].

In general, intermolecular interactions play a prominent role in the solid state[24] and structural studies in microcrystalline proteins or amyloid fibrils have employed dedicated labeling patterns that separate polarization transfer dynamics due to intra – or intermolecular transfer[24] and the quenching thereof[25]. Indeed, mixing molecular species in different labeling patterns furthermore offers a route to probe intermolecular contacts in ssNMR[4,26]. In membranes, additional interactions involving the lipid-protein interface or surrounding water can be used to infer molecular orientation and global structure (see, e.g., Ref. [27,28]) and, at the same time, reduce spectral congestion.

Spectral simplification furthermore can be obtained using mobility filters[29] that separate signals sets of mobile and rigid protein components. Similar to the solution state, an additional reduction in spectral complexity may be obtained using paramagnetic quenchers and ^{1}H/^{2}H exchange experiments. In addition to the study of molecular motion, protein deuteration has been demonstrated to significantly enhance the possibilities to include proton evolution and detection dimensions in MAS-based solid-state NMR experiments. Such approaches have been useful to establish structural constraints of solid-phase proteins[30,31,32] or to characterize protein-water interactions using multi-dimensional ssNMR methods[33]. With increasing levels of deuteration, impressive improvements in ^{1}H line width have been reported[34]. Yet, protein deuteration often reduces protein expression levels, influences ssNMR resonance frequencies and CP efficiencies and compromises the possibility to probe structurally relevant proton-proton distance constraints. As a result, ssNMR applications to complex biomolecules have thus far been limited. In the future,

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the combination of fractionally deuterated biomolecules, ultra-high speed MAS and the use of dedicated multiple-pulse schemes may provide a compromise between enhanced ^{1}H resolution and structural information.

Integrated approaches

Clearly, ssNMR provides a rich source of structural and dynamical information, even if molecules become larger and additional studies are necessary to streamline the determination of molecular structure and dynamics by ssNMR methods. At the same time, advances in other research areas such as theoretical chemistry and molecular modeling are taking place. These developments along with the increasing utility of other biophysical techniques strongly suggest that future biomolecular applications of ssNMR will profit from applying hybrid concepts to solve a challenging problem in structural biology or material science.

Already, the ability to predict the ssNMR shift from first principles or using hybrid strategies has changed the ways in which (isotropic and anisotropic) chemical-shift information is used. In proteins, the increasingly accurate correlation between ssNMR chemical shift and structure[35] can be used to assess secondary structure or estimate structural changes. Other integrated approaches may combine NMR and molecular dynamics or modelling. For example, combining ssNMR, solution-state NMR and in silico modeling, we recently characterized structural and functional aspects of a 400 aa protein complex in membranes[23]. In these experiments, the judicious choice of the amino-acid labeling pattern was crucial to provide sufficient spectral resolution. It seems likely that such studies, together with the application of three – or even higher-dimensional ssNMR correlation experiments (see, e.g., [11,12]) will improve the prospects to study large biomolecules under functionally relevant conditions.

Outlook

Post-genomic research efforts, high-throughput methodology and advances in areas such as mass spectrometry or electron microscopy have revealed that biological functioning is controlled by biomolecular interaction networks, often in a heterogeneous and dense molecular environment. For example, the cellular response to outside stimuli such as light or nutrients or the process of protein aggregation in the context of Alzheimer's or Parkinson's disease are taking place in a more complex and dense cellular environment than previously envisioned. To understand these fundamental processes at atomic resolution and restore them in a pharmacological context, structural biology tools are needed that can be applied in a complex molecular environment. SsNMR clearly has made progress to address such systems on the molecular level. At the same time, ssNMR can probe a large dynamic range, giving insight into molecular processes that take place from the time frame of nanoseconds to seconds.

With increasing molecular complexity, both spectroscopic sensitivity and resolution are of critical importance. Recently, exciting concepts that aim at enhancing ssNMR sensitivity have been described. These range from combining paramagnetic doping and ultra-fast Magic Angle Spinning (MAS)[36] to the widespread application of Dynamic Nuclear Polarization (DNP)[37]. Such techniques will spark the development of additional sample preparation routes. For example, the combination of isotope and paramagnetic labeling, the introduction of non-natural amino acids or the tailored use of polarization agents will provide new possibilities to study biomolecules of increasing complexity.

At the same time, advancements in ssNMR methodology and instruments are likely to push the current boundary conditions of biomolecular ssNMR. Proteoliposomal complexes, cellular extracts, whole-cell preparations or tissue samples are just a few of the potential areas that ssNMR may be able to tackle in the future. Clearly, the prospects for ssNMR as a biomolecular tool to bridge the gap between traditional structural biology and cell biology are exciting and, without doubt, state-of-the-art sample preparation methods will be of vital relevance to realize such goals in the future.

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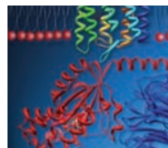

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Products for Uniform Labeling

Minimal media products are the foundation for uniformly labeling proteins for Solid-State NMR experiments. They provide a relatively simple and cost-effective means to incorporate either ^{13}C, ^{15}N, and D or various combinations of these isotopes. By utilizing these tools alone, researchers are able to obtain a vast amount of structural information leading to almost complete resonance assignments. Isotec offers all of the labeled minimal media products in the convenient sizes below or in larger bulk quantities upon request.

Minimal Media Products

Cat. No.	Name	Isotopic Purity
299251-1G 299251-10G 299251-20G	Ammonium-^{15}N chloride	98 atom % ^{15}N
594091	Ammonium-$^{15}N,d_4$ deuteroxide solution	99 atom % ^{15}N, 98 atom % D
488011-5G 488011-10G	Ammonium-^{15}N hydroxide solution, ~3 N in H_2O	98 atom % ^{15}N
299286-10G 299286-20G	Ammonium-$^{15}N_2$ sulfate	98 atom % ^{15}N
151882-1KG 151882-1.107KG	Deuterium oxide	99.9 atom % D
617385-1KG 617385-1.107KG	Deuterium oxide	99.8 atom % D
552003-1G 552003-10G	D-Glucose-1,2,3,4,5,6,6-d_7	97 atom % D
616338-250MG	D-Glucose-d_{12}	97 atom % D
389374-1G 389374-2G 389374-3G 389374-10G	D-Glucose-$^{13}C_6$	99 atom % ^{13}C

Cat. No.	Name	Isotopic Purity
552151-1G 552151-5G	D-Glucose-$^{13}C_6$,1,2,3,4,5,6,6-d_7	99 atom % ^{13}C, 97 atom % D
447498-1G 447498-5G	Glycerol-d_8	98 atom % D
489476-500MG	Glycerol-$^{13}C_3$	99 atom % ^{13}C
669024-500MG	Glycerol-$^{13}C_3$, d_8	99 atom % ^{13}C, 98 atom % D
176079-5G 176079-25G	Sodium acetate-d_3	99 atom % D
282014-250MG 282014-1G	Sodium acetate-$^{13}C_2$	99 atom % ^{13}C
299111-100MG 299111-500MG	Sodium acetate-$^{13}C_2,d_3$	99 atom % ^{13}C, 99 atom % D
373842-1G 373842-5G	Sodium formate-d	99 atom % D
488356-5G	Succinic acid-d_6	98 atom % D
491985-100MG	Succinic acid-$^{13}C_4$	99 atom % ^{13}C

ISOGRO® Complex Growth Media

While minimal media products are the basic tools to facilitate the incorporation of a uniform isotopic label for Solid-State NMR experiments, there are potential problems which may arise when relying on this method exclusively. There can be difficulties expressing sufficient quantities of certain proteins while also experiencing significant lag times in growth periods. To avoid these problems, Isotec offers an algal lysate derived complex growth media, ISOGRO. This product is highly effective at isotopic label incorporation as well as enhancing protein expression and can be utilized in two primary manners: as a stand-alone media or as a supplement to M9 minimal media.[1-3]

ISOGRO as a Stand-alone Media

For optimal results, incorporate 10g of ISOGRO per Liter of culture.

- Improve recombinant protein yields up to 80% compared to commercially available complex growth media "B" (Figure 1)
- Substantially increase recombinant protein expression levels using ISOGRO versus M9 media
- Save time by using ISOGRO growth media to shorten production time

ISOGRO as a Supplement to M9 Media

Supplement M9 media with as little as 1g of ISOGRO per Liter of culture.

- Decrease lag time by as much as 60% (Figure 2)
- Maximize OD and recombinant protein expression
- Improve the production of difficult to express proteins in *E. coli*

As a standard quality control measure, the suitability of each batch of ISOGRO as a culture medium is determined by comparison with an LB growth curve.

Cat. No.	Name	Isotopic Purity
606863-1G	ISOGRO-^{13}C Powder Growth Medium	99 atom % ^{13}C
616729-1G	ISOGRO-D Powder Growth Medium	97 atom % D
606871-1G	ISOGRO-^{15}N Powder Growth Medium	98 atom % ^{15}N
606839-1G	ISOGRO-^{13}C,^{15}N Powder Growth Medium	99 atom % ^{13}C, 98 atom % ^{15}N
608300-1G	ISOGRO-^{15}N,D Powder Growth Medium	98 atom % ^{15}N, 97 atom % D
608297-1G	ISOGRO-^{13}C,^{15}N,D Powder Growth Medium	99 atom % ^{13}C, 98 atom % ^{15}N, 97 atom % D

ISOGRO as a Stand-Alone Media

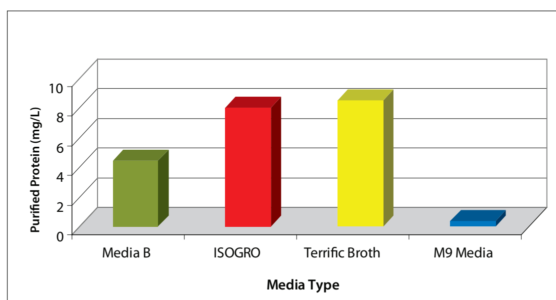

Figure 1. The final yield of purified recombinant protein derived from each liter of culture. Acknowledgement: Date provided by Dr. Ross Overman and Dr. Kevin Embry, AstraZeneca, U.K.

ISOGRO as a Supplement to M9 Media

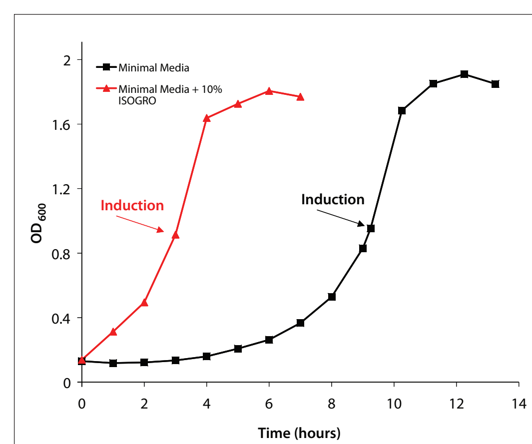

Figure 2. Data provided by Dr. Paul Rosevear, The Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Center, Cincinnati, Ohio.

For detailed ISOGRO protocols visit, ***aldrich.com/bionmr***

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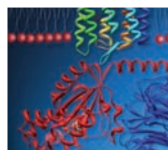
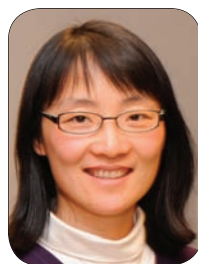


Isotopic Labeling for NMR Spectroscopy of Biological Solids



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Isotopic labeling plays an indispensable role in structure determination of proteins and other biomacromolecules using solid-state NMR. It not only enhances the NMR sensitivity but also allows for site-specific interrogation of structures and intermolecular contacts. This article gives a survey of the different isotopic labeling approaches available today for biological solid-state NMR research.

Biosynthetic uniform ^{13}C, ^{15}N labeling

The simplest and most cost-effective biosynthetic labeling method for protein solid-state NMR is to uniformly label all carbon and nitrogen atoms with ^{13}C and ^{15}N. In this way, a single protein sample can in principle provide all the structural constraints – dihedral angles and distances - about the protein. The labeled precursors are typically uniformly (U) ^{13}C-labeled glucose or glycerol, and ^{15}N-labeled ammonium chloride or ammonium sulfate. These compounds can be readily incorporated into the growth media for protein expression. Uniform ^{13}C, ^{15}N-labeling has seen the most widespread application in the development of new magic-angle-spinning (MAS) multidimensional correlation techniques for full structure determination of proteins. A number of microcrystalline proteins whose structures are known from X-ray crystallography or solution NMR have been used to demonstrate the ability of solid-state NMR to obtain de novo three-dimensional structures. These microcrystalline proteins include ubiquitin [1,2], GB1 [3,4], thioredoxin [5], and the a-spectrin SH3 domain [6]. Uniform ^{13}C and ^{15}N labeling has also been used effectively in structure determination of amyloid fibril proteins, such as transthyretin [7], the HET-s prion protein [8], and a human prion protein [9]. A common feature of the proteins amenable to this labeling scheme is that they possess sufficient structural order on the nanometer scale to give highly resolved spectra. Without this high conformational homogeneity and the resulting high spectral resolution, uniform ^{13}C labeling is not recommended since it would cause considerable spectral congestion. Various 2D, 3D [1,10,11], and 4D [12] correlation techniques have been developed to resolve the signals of uniformly ^{13}C, ^{15}N-labeled proteins and to determine internuclear distances and dihedral angles.

Uniform ^{13}C and ^{15}N labeling has also been applied to a handful of membrane proteins, such as potassium ion channels [13], seven-transmembrane-helix proteins [14,15], light-harvesting complexes[16], membrane-bound enzymes [17], and bacterial toxins [18]. Since membrane proteins usually have larger conformational disorder than microcrystalline proteins or fibril-forming proteins, the spectral resolution of membrane proteins is generally lower. Nevertheless, detailed structural information of key regions of these membrane proteins or the global topology of membrane proteins in the lipid bilayer, such as their depth of insertion, could still be obtained even using uniformly ^{13}C, ^{15}N-labeled samples.

The main spectroscopic challenges involved in MAS NMR of uniformly ^{13}C-labeled proteins are three-fold: 1) the limited dispersion of ^{13}C isotropic chemical shifts given the inhomogeneous linewidths of the sample; 2) the ^{13}C-^{13}C scalar couplings that contribute to line broadening; and 3) the dipolar truncation effect that makes it difficult to measure long-range ^{13}C-^{13}C distances in the presence of strong one-bond ^{13}C-^{13}C dipolar couplings. Static ^{15}N NMR of oriented membrane peptides and proteins do not have these challenges, since the spectral dispersion is determined by the much larger anisotropic chemical shift range rather than the isotropic chemical shift range, and because there is no ^{15}N-^{15}N scalar coupling nor any sizeable ^{15}N-^{15}N dipolar coupling in proteins. Therefore, uniform ^{15}N labeling entails few complications for orientation determination of membrane proteins and indeed has seen fruitful applications [19,20]. On the other hand, it is clearly desirable to increase the information content of the aligned sample spectra by including ^{13}C dimensions. New spectroscopic challenges need to be overcome in ^{13}C NMR of oriented membrane proteins. For example, ^{13}C-^{13}C dipolar couplings of U-^{13}C-labeled proteins are no long removed by MAS in these static samples. Strategies for decoupling the ^{13}C-^{13}C couplings and for correlation experiments under the static condition have been proposed and demonstrated on single crystal model compounds [21]. Random fractional ^{13}C labeling, which strikes a compromise between resolution and structural information, has also been proposed [22].

Products for Selective ^{13}C Labeling

Cat. No.	Name	Isotopic Purity
492639-250MG	Glycerol-1,3-$^{13}C_2$	99 atom % ^{13}C
489484	Glycerol-2-^{13}C	99 atom % ^{13}C
297046-250MG 297046-1G 297046-10G	D-Glucose-1-^{13}C	99 atom % ^{13}C
310794-250MG 310794-1G	D-Glucose-2-^{13}C	99 atom % ^{13}C
453196-100MG 453196-250MG	D-Glucose-1,6-$^{13}C_2$	98 atom % ^{13}C
605506	D-Glucose-2,5-$^{13}C_2$	99 atom % ^{13}C
490733-250MG	Sodium pyruvate-3-^{13}C	99 atom % ^{13}C
485349-500MG	Succinic acid-1,4-$^{13}C_2$	99 atom % ^{13}C
488364-100MG	Succinic acid-2,3-$^{13}C_2$	99 atom % ^{13}C

Biosynthetic selective ^{13}C labeling

Two of the three challenges listed above for studying U-^{13}C labeled proteins are nicely addressed by the complementary approach of selective ^{13}C labeling. In this approach, carbon precursors that contain only specific ^{13}C-labeled sites are incorporated into the protein expression media. These labeled sites are converted, through well-known enzymatic pathways [23], to predictable positions in the twenty amino acids, which result in selectively and extensively labeled proteins. All residues of the same amino acid type have the same labeled positions, but different amino acids have different labeled positions due to their distinct enzymatic pathways.

The two main precursors that have been demonstrated are [2-^{13}C] glycerol, which primarily label the Cα carbons of amino acids, and [1,3-^{13}C] glycerol, which label the other sites skipped by [2-^{13}C] glycerol. Each precursor tends to label alternating carbons, thus removing any sizeable ^{13}C-^{13}C scalar couplings and the trivial one-bond dipolar couplings. This selective labeling approach was originally proposed by LeMaster and Kushlan for solution NMR studies and subsequently adopted for solid-state NMR [24-26]. By far the most important application of selective ^{13}C labeling is distance extraction from ^{13}C-^{13}C correlation spectra. Other amino acid precursors can in principle also be exploited, for example, oxaloacetate, a-ketoglutarate, and pyruvate, as having been done in protein solution NMR. In addition, ^{13}C-labeled carbon dioxide has been used for studying plant cell wall proteins [27,28].

Reverse labeling: combining biosynthetic labeling with unlabeled amino acids

Another strategy to reduce the spectral congestion without resorting to amino-acid-specific labeling is to combine a labeled general carbon precursor with unlabeled amino acids, so that only a subset of amino acid types will be labeled. For membrane protein structural studies, one version of this strategy is the **TEASE** (**te**n-**a**mino-acid-**s**elective-and-**e**xtensive) labeling protocol [25]. In this approach, [2-^{13}C] glycerol and ten unlabeled amino acids serve as the carbon precursors of the expression media. The ten amino acids are Glu, Gln, Pro, Arg, Asp, Asn, Met, Thr, Ile, and Lys, which are products of the citric acid cycle. Normally, the cycle distributes the ^{13}C labels in glucose or glycerol to produce fractionally labeled sites in these amino acids, so that their signals are more difficult to assign in the NMR spectra than amino acids synthesized from the glycolysis pathway. Due to the approximate hydrophobic versus hydrophilic distinction of the amino acids from the glycolysis pathway versus the citric acid cycle, a membrane protein could in principle be TEASE ^{13}C-labeled to selectively detect the transmembrane segments rich in the hydrophobic residues.

Clearly, this reverse labeling approach is highly flexible and can be adapted for different applications. For example, a U-^{13}C-labeled precursor can be combined with a small set of unlabeled amino acids that are dominant in the protein. Unlabeling of these amino acid types simplifies the NMR spectra considerably[14], and does not bring any disadvantages to the protein expression.

Site-specific labeling of synthetic peptides and proteins

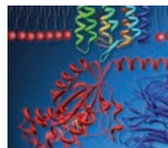
Site-specific ^{13}C and ^{15}N labeling continues to provide rich structural information about polypeptides that are too small to be recombinantly expressed or proteins that are too large for uniformly ^{13}C-labeled spectra to be analyzable. For polypeptides shorter than 40 amino acids, chemical synthesis is generally feasible, therefore ^{13}C, ^{15}N-labeled amino acids in their protected forms can be incorporated into the peptide synthesis for site-specific labeling.

A common site-specific amino acid labeling strategy is the scattered uniform ^{13}C, ^{15}N-labeling of residues. As long as the yield of the peptide synthesis is not prohibitively low, the combination of several samples with different U-^{13}C, ^{15}N-labeled residues can eventually map out the complete structure of the polypeptide of interest. This approach has been used extensively to study amyloid peptides [29] and membrane peptides [30-32]. Non-uniform ^{13}C and ^{15}N labeling of specific amino acid residues has also been applied. The most commonly labeled sites are the ^{13}CO of the polypeptide backbone, and sometimes the sidechain ^{15}N of lysine residues. Applications usually involve distances measurements using heteronuclear REDOR [33] or homonuclear ^{13}C recoupling [34] experiments.

Since most peptides are synthesized using the Fmoc solid phase chemistry, site-specific amino acid labeling requires Fmoc-protected amino acids. For hydrophobic amino acids, their Fmoc protected forms are usually commercially available and can also be synthesized readily from their unprotected forms. On the other hand, polar amino acids require both backbone and sidechain protection, thus are more costly and difficult to prepare. While Fmoc solid-phase synthesis is the dominant chemistry in peptide synthesis, t-Boc solid-phase synthesis has also been used for interesting structure determination targets [35]. Boc-protected ^{13}C, ^{15}N-labeled amino acids are so far much less common. Therefore, increased commercial production and availability of t-Boc-protected amino acids are desirable.

Other isotopic labels for studying macromolecular complexes and protein chemistry

For large macromolecular complexes such as the cell walls of plants and bacteria, and for membrane proteins bound to ligands or inhibitors, it is often important to increase the diversity of isotopic labeling to enable intermolecular distance measurements. Two isotopes are readily available for this purpose: ^{2}H and ^{19}F. ^{19}F is naturally 100% abundant and has a long history of being incorporated into amino acids [36-38] as well as non-peptidic molecules such as lipids and pharmaceutical drugs [39]. Site-specific ^{2}H labeling is most commonly used for methyl groups of Ala, Leu, and Val, and is an excellent probe of the dynamics



of proteins [40,41] and DNA [42]. More recently, perdeuteration of proteins in combination with uniform ^{13}C and ^{15}N labeling has been exploited as a means to obtain high-resolution spectra of proteins, as perdeuteration removes ^{1}H dipolar coupling as a line broadening mechanism. The back-exchanged proteins have ^{1}H spins only at exchangeable positions such as the amide hydrogens and lysine amino groups. These sparse protons can be used as a high-sensitivity detection nucleus. Perdeuterated microcrystalline proteins have been used to study relaxation dynamics of proteins and protein-water interactions [43-45].

To produce $^{13}C/^{15}N/^{2}H$ triply labeled recombinant proteins, one needs to use ^{2}H and ^{13}C labeled glucose, which is commercially available. The main challenge in this type of protein expression is for the cells to tolerate a water-deuterated liquid culture, which usually decreases the protein expression yield.

Future prospects

Isotopic labeling is an essential and versatile tool for NMR structural biology. Creative labeling of NMR-sensitive nuclei (^{13}C, ^{15}N, and ^{2}H), combined with strategic exploitation of naturally 100% abundant nuclei such as ^{19}F and ^{31}P, can advance the structural biology of many insoluble macromolecules important in biology.

For future progress in solid-state NMR structural biology, it will be important to develop a more diverse panel of isotopically labeled compounds and to produce the existing compounds at a more economical level. Since biosynthetically obtained ^{13}C-labeled precursors are ubiquitous and relatively simple to produce, one of the future challenges is a chemical one, which is to produce a diverse array of specifically labeled specifically labeled amino acids and other small biomolecules with isotopic labels at desired positions.

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Protected Amino Acids for Peptide Synthesis

Protected amino acids allow for the precise control of the position of labeled amino acids within a peptide of interest which allows researchers to address structural questions. This type of tool can be extremely beneficial in the analysis of membrane proteins, self associating proteins forming insoluble deposits, and macromolecular structures. We offer a wide selection of both Fmoc and t-Boc protected amino acids for this application. Visit *aldrich.com/protectedaa* for a complete listing.

				Fmoc Protected		t-Boc Protected	
Amino Acid			Formula	^{15}N	^{13}C, ^{15}N	^{15}N	^{13}C, ^{15}N
L-Alanine	A	Ala	$C_3H_7NO_2$	489905	667064	489913	485837
L-Arginine	R	Arg	$C_6H_{14}N_4O_2$		653659[P]		
L-Asparagine	N	Asn	$C_4H_8N_2O_3$	579890 668745[T]	658936[T]		
L-Aspartic Acid	D	Asp	$C_4H_7NO_4$	492906	683639[O]	588792	
L-Cysteine	C	Cys	$C_3H_7NO_2S$	676608[T]			
L-Glutamic Acid	E	Glu	$C_5H_9NO_4$	490008	666009[O]	587699	588407[Z]
L-Glutamine	Q	Gln	$C_5H_{10}N_2O_3$	703109[T]	663956[T]	587702	
Glycine	G	Gly	$C_2H_5NO_2$	485756	489530	486701	587737
L-Histidine	H	His	$C_6H_9N_3O_2$	676969[T]	707295[T]		
L-Isoleucine	I	Iso	$C_6H_{13}NO_2$	578622	597228		
L-Leucine	L	Leu	$C_6H_{13}NO_2$	485950	593532	492930	
L-Lysine	K	Lys	$C_6H_{14}N_2O_2$	577960[B]	653632[B]		
L-Methionine	M	Met	$C_5H_{11}NO_2S$	609196	653640		
L-Phenylalanine	F	Phe	$C_9H_{11}NO_2$	609072	651443	486833	
L-Proline	P	Pro	$C_5H_9NO_2$	589519	651451		
L-Serine	S	Ser	$C_3H_7NO_3$	609145[O]	658928[O]		
L-Threonine	T	Thr	$C_4H_9NO_3$	658162[O]	694274[O]		672866[Z]
L-Tryptophan	W	Trp	$C_{11}H_{12}N_2O_2$	648302	718696[B]		
L-Tyrosine	Y	Tyr	$C_9H_{11}NO_3$	658901[O]	658898[O]	591092	
L-Valine	V	Val	$C_5H_{11}NO_2$	486000	642886	486019	

Secondary protection groups: [P]PBF, [O]O-t-Butyl, [B]t-Boc, [T]trityl, [Z]O-Benzyl

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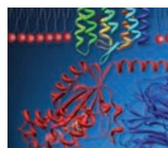
Uniformly Labeled Amino Acids

Uniformly labeled amino acids can be used to incorporate various labeling patterns when used with minimal media, complex growth media, or in cell-free protein expression systems. This type of labeling offers researchers flexibility in achieving their desired labeling pattern. In addition to the uniformly labeled amino acids below, Isotec has an extensive offering of selectively labeled amino acids which can be viewed at *aldrich.com/aminoacids*

Amino Acid			Formula	D	^{15}N	^{13}C	^{13}C, ^{15}N
L-Alanine	**A**	**Ala**	$C_3H_7NO_2$	485845[d]	332127	489875	489883
L-Arginine	**R**	**Arg**	$C_6H_{14}N_4O_2$		600113	643440	608033
L-Asparagine	**N**	**Asn**	$C_4H_8N_2O_3$	672947	485918	588695	608157
L-Aspartic Acid	**D**	**Asp**	$C_4H_7NO_4$	489980[d]	332135	604852	607835
L-Cysteine	**C**	**Cys**	$C_3H_7NO_2S$	701424[d]	609129		658057
L-Glutamic Acid	**E**	**Glu**	$C_5H_9NO_4$	616281[d]	332143	604860	607851
L-Glutamine	**Q**	**Gln**	$C_5H_{10}N_2O_3$	616303[d]	490032	605166	607983
Glycine	**G**	**Gly**	$C_2H_5NO_2$	175838	299294	283827	489522
L-Histidine	**H**	**His**	$C_6H_9N_3O_2$		574368	722871	608009
L-Isoleucine	**I**	**Iso**	$C_6H_{13}NO_2$		609013		608092
L-Leucine	**L**	**Leu**	$C_6H_{13}NO_2$	492949[d]	340960	605239	608068
L-Lysine	**K**	**Lys**	$C_6H_{14}N_2O_2$		609021	643459	608041
L-Methionine	**M**	**Met**	$C_5H_{11}NO_2S$		609242		608106
L-Phenylalanine	**F**	**Phe**	$C_9H_{11}NO_2$	490148[d]	490105		608017
L-Proline	**P**	**Pro**	$C_5H_9NO_2$		608998	604801	608114
L-Serine	**S**	**Ser**	$C_3H_7NO_3$		609005	604887	608130
L-Threonine	**T**	**Thr**	$C_4H_9NO_3$		609099	677604	607770
L-Tryptophan	**W**	**Trp**	$C_{11}H_{12}N_2O_2$		574600		574597
L-Tyrosine	**Y**	**Tyr**	$C_9H_{11}NO_3$		332151	492868	607991
L-Valine	**V**	**Val**	$C_5H_{11}NO_2$	486027[d]	490172		600148

[d]Only non-exchangeable positions are deuterated



α-Ketoacids

α-Ketoacids for Selective Methyl Labeling

The use of labeled α-Ketoacids has been invaluable for enabling the solution NMR studies of progressively larger proteins and supra-molecular systems[1-3]. These products allow for enhanced sensitivity and resolution by incorporating selective ^{13}C and/or D labels into the methyl groups of the highly abundant residues of Leucine, Valine, and Isoleucine. While initial applications have centered on solution NMR, there remains potential to exploit these labeling patterns to explore more challenging proteins and protein-complexes by Solid-State NMR.

For additional information on α-Ketoacids along with a technical article written by Dr. Lewis Kay and Dr. Vitali Tugarinov, visit *sigma-aldrich.com/bionmr*

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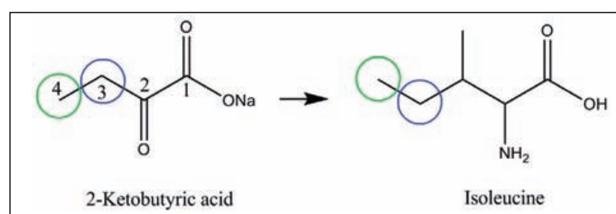

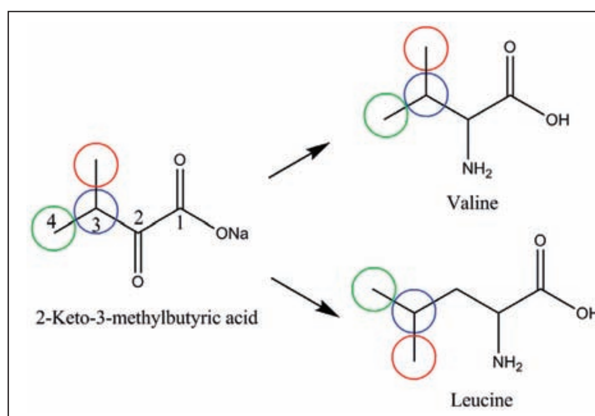

2-Ketobutyric acid

Cat. No.	Name	Isotopic Purity
717150-250MG	2-Ketobutyric acid-3, 3-d_2, sodium salt hydrate	97 atom % D
571342-250MG	2-Ketobutyric acid-4-^{13}C sodium salt hydrate	99 atom % ^{13}C
589276-100MG	2-Ketobutyric acid-4-^{13}C, 3, 3-d_2 sodium salt hydrate	99 atom % ^{13}C, 98 atom % D
634727-500MG	2-Ketobutyric acid-4-^{13}C, 4, 4-d_2 sodium salt hydrate	99 atom % ^{13}C, 98 atom % D
637831-1G	2-Ketobutyric acid-4-^{13}C, 4-d_1 sodium salt hydrate	99 atom % ^{13}C, 97 atom % D
607533-100MG	2-Ketobutyric acid-4-^{13}C, 3, 3, 4,4, 4-d_5 sodium salt hydrate	97 atom % D (CD_2), 99 atom % ^{13}C, 50-70 atom % D($^{13}CD_3$)
607541-100MG	2-Ketobutyric acid-$^{13}C_4$, 3, 3-d_2 sodium salt hydrate	99 atom % ^{13}C, 98 atom % D

2-Keto-3-methylbutyric acid

Cat. No.	Name	Isotopic Purity
571334-100MG	2-Keto-3-(methyl-^{13}C)-butyric acid-4-^{13}C sodium salt	99 atom % ^{13}C
634379-250MG	2-Keto-3-(methyl-$^{13}C,d_2$)-butyric acid-4-$^{13}C,d_2$ sodium salt	98 atom % ^{13}C, 98 atom % D
596418-100MG	2-Keto-3-(methyl-d_3)-butyric acid-1,2,3,4-$^{13}C_4$ sodium salt	99 atom % ^{13}C, 98 atom % D
637858-250MG	2-Keto-3-(methyl-d_3)-butyric acid-1,2,3,4-$^{13}C_4$, 3-d_1 sodium salt	99 atom % ^{13}C, 98 atom % D
594903-100MG	2-Keto-3-(methyl-d_3)-butyric acid-4-^{13}C sodium salt	99 atom % ^{13}C, 98 atom % D
589063-100MG	2-Keto-3-(methyl-^{13}C)-butyric-4-^{13}C, 3-d acid sodium salt	99 atom % ^{13}C, 98 atom % D
691887	2-Keto-3-(methyl-d_3)-butyric acid-4-^{13}C, 3-d_1 sodium salt	99 atom % ^{13}C, 97 atom % D
607568-250MG	2-Keto-3-methylbutyric acid-$^{13}C_5$, 3-d_1 sodium salt	99 atom % ^{13}C, 98 atom % D
663980	2-Keto-3-methylbutyric acid-$^{13}C_5$ sodium salt	99 atom % ^{13}C
717169-250MG	2-Keto-3-methylbutyric-3-d acid, sodium salt hydrate	98 atom % D

Labeled Ubiquitin Protein Standards

Isotec now offers high quality human Ubiquitin in a wide variety of labeling patterns. Labeled Ubiquitin allows researchers to develop new methodologies for Solid-State NMR analysis[1], perform studies pertaining to molecular motion[2], and to verify NMR instrumentation and probe performance. Our Ubiquitin is supplied as a lyophilized powder and does not contain a His-tag. To ensure the highest quality, each batch is analyzed by NMR, Mass Spectrometry, and SDS-PAGE.

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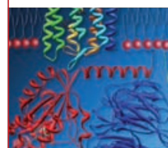
Cat. No.	Name	Isotopic Purity
709409-5MG 709409-10MG	Ubiquitin-^{15}N	98 atom % ^{15}N
709441-5MG 709441-10MG	Ubiquitin-^{15}N,D	98 atom % ^{15}N, 97 atom % D
709468-5MG 709468-10MG	Ubiquitin-$^{13}C,^{15}N$	99 atom % ^{13}C, 98 atom % ^{15}N
709395-5MG 709395-10MG	Ubiquitin-$^{13}C,^{15}N$,D	99 atom % ^{13}C, 98 atom % ^{15}N, 97 atom % D
709417-5MG 709417-10MG	Ubiquitin-unlabeled	NA



Additional Products for Solid-State NMR

Solid-State NMR applications are continually expanding and now cover a diverse range of inorganic materials. Improvements in hardware and software combined with the commercial availability of various isotopes have accelerated structural research in the areas such as:

- Glasses
- Cements
- Semiconductors
- Foods
- Polymers
- Minerals
- Ceramics
- Metals
- Surfaces
- Inorganic complexes

Isotec offers a wide range of products to meet the needs of these areas of research. In addition to providing some of the basic compounds for isotope incorporation such as Nitrogen-^{15}N gas, Deuterium gas, Carbon-^{13}C monoxide, Water-^{17}O, we also offer labeled monomers and polymers in a variety of labeling patterns.

To view all of our stable isotope compounds visit, our online product catalog at *aldrich.com/sicatalog*

Cat. No.	Name	Isotopic Purity
606421	Acrolein-2-^{13}C	99 atom % ^{13}C
487899-250MG	Acrylic acid-1-^{13}C	99 atom % ^{13}C
586641	Acrylonitrile-$^{13}C_3$	99 atom % ^{13}C
489697	Adipic acid-1,6-$^{13}C_2$	99 atom % ^{13}C
451835-1G	Bisphenol A-d_{16}	98 atom % D
530549-5G	Ethylene glycol-d_6	98 atom % D
489360-1G	Ethylene glycol-$^{13}C_2$	99 atom % ^{13}C
444960	Methyl methacrylate-d_8	99 atom % D
602841	Oxygen-$^{17}O_2$ gas	90 atom % ^{17}O
490504	Phenol-$^{13}C_6$	99 atom % ^{13}C
487007	Poly(ethylene-d_4)	98 atom % D
606545	Styrene-α-^{13}C	99 atom % ^{13}C
609862	Water-^{17}O	90 atom % ^{17}O

Additional Literature of Interest:

1. Spiess H.W.; *J. Polym. Sci. Part A: Polym. Chem.* **2004**, 42, 5031-5044.
2. Groves W.R.; and Pennington C.H., *Chemical Physics* **2005**, 315, 1-7.
3. Garvais C.; Babonneau F.; Ruwisch L.; Hauser R.; and Riedel, R. *Can. J. Chem* **2003**, 81, 1359-1369.

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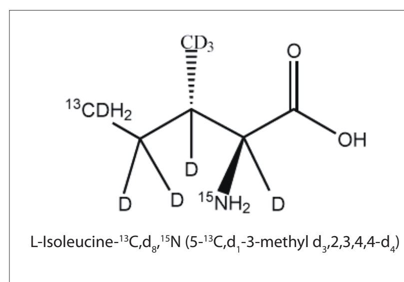

L-Isoleucine-$^{13}C,d_8,^{15}N$ (5-$^{13}C,d_1$-3-methyl d_3,2,3,4,4-d_4)

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