

# The Synthesis

Cambridge Isotope Laboratories



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## STABLE ISOTOPE LABELING *in Proteomics*

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Proteomics, the analysis of the proteins expressed by a cell, tissue, or organism under a specific set of conditions, has undergone a tremendous period of growth in the past few years. Proteomic studies are typically designed to analyze hundreds or thousands of proteins in a single analysis and aim to provide a global view of changes in protein expression that occur in different cellular growth states or when the cell is treated with a given agent or regimen. While proteomics is formally defined as the complete characterization of the protein complement of a cell, including post-translational modifications, a great deal of intellectual effort has been focused on developing methods to globally measure changes in relative protein abundances between two distinct cell systems (i.e., control vs. treated).

The driving technology supporting proteomic investigations has been mass spectrometry (MS). The ability to rapidly identify proteins and its high sensitivity are just two of the key features of MS that has made it invaluable in proteomics. While changes in protein expression have typically been studied by first separating samples of interest on two distinct two-dimensional polyacrylamide gels (2D-PAGE) followed by comparing the intensity of the Coomassie or silver-stained spots between gels, this method has many deficiencies related to reproducibility, proteome coverage, and quantitation. Fortunately, there have been several recent developments in the use of stable isotope labeling strategies that allow combined, yet isotopically distinct, proteome samples (from different sources) to be analyzed.

While mass spectrometry has not been historically used for measuring relative protein abundances, developments in the area of stable isotope labeling are now making this scenario feasible at both the intact protein and peptide level [1,2]. One of the earliest demonstrations of isotopic labeling strategies for whole proteomes was the analysis of intact proteins to examine the cadmium ( $\text{Cd}^{2+}$ ) stress response in *Escherichia coli*. In these studies, *E. coli* was grown in both normal (i.e. natural isotopic abundance) and rare-isotope ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ) depleted media [1]. Changes in relative protein abundances were measured by removing equal aliquots of cells from the unstressed (normal medium) and stressed (depleted medium) cultures at different time intervals after  $\text{Cd}^{2+}$ -addition, mixing them prior to sample processing and the extracted proteins were analyzed by capillary isoelectric focusing coupled on-line with Fourier transform

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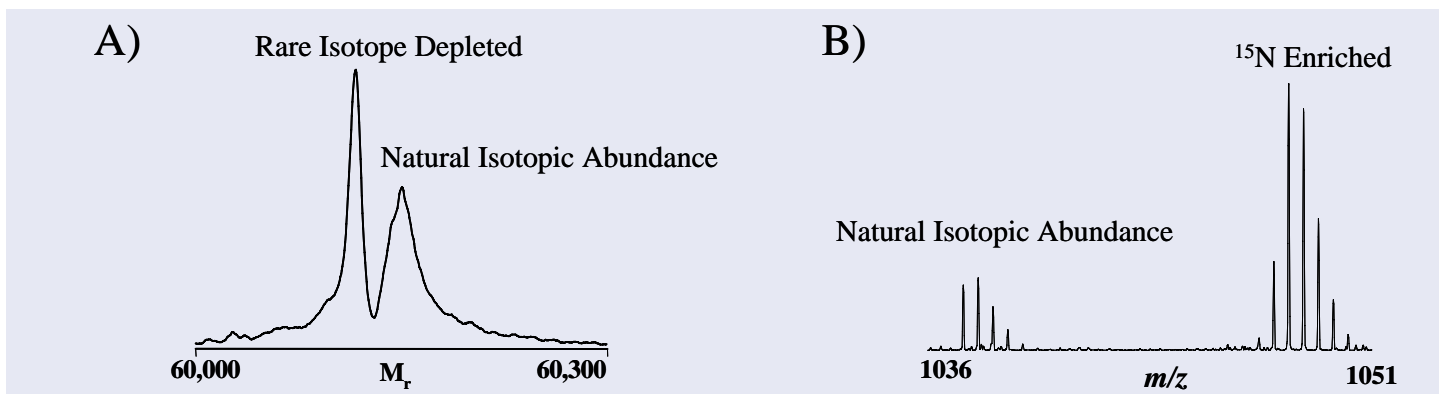


Figure 1

Examples of stable-isotope labeling of an (A) intact protein and (B) peptide observed in the MS analysis of an *E. coli* and *Deinococcus radiodurans* proteome samples, respectively. The two isotopic versions of each were obtained by culturing the cells separately in normal and either isotopically depleted (A) or  $^{15}\text{N}$ -enriched (B) media. Combining the two separate cultures provides two isotopic versions for every species present in the samples.

ion cyclotron resonance (FTICR) MS. In other stable isotope-labeling approaches, cells have been cultured in  $^{15}\text{N}$ -enriched medium and combined with cells cultured in normal medium and changes in relative abundance measured by analyzing the peptides produced from a proteolytic digestion of intact proteins [2]. In both of these metabolic labeling methods, two isotopically distinct versions of each protein (or peptide) are observed and the relative abundance of the specific protein is quantified by comparing observed peak intensities of each species in the mass spectra, as shown in Figure 1.

While the metabolic labeling method described above is limited to cells that can be cultured in specifically formulated media, other isotope labeling methods have been developed that are applicable to proteome samples isolated from any conceivable source. One of the most exciting developments in the use of stable isotope labeling to quantify changes in the expression of proteins in proteome studies is the isotope-coded affinity tags (ICAT) method [3]. In the application of ICAT labeling, shown in Figure 2, proteins are modified with a cysteine-specific reactive group that covalently modifies reduced Cys residues. The ICAT reagent also contains a biotin tag, allowing the specific isolation of the modified Cys-containing peptides using immobilized avidin. Changes in the relative abundance of peptides from distinct proteome samples is accomplished by the use of isotopically distinct versions of the ICAT reagent; a light isotopic version and a heavy isotopic version in which eight protons in the linker region between the thiol reactive group and the biotin moiety of the ICAT reagent have been substituted with eight deuterons. ICAT labeling results in both stable-isotope labeled Cys-polypeptides, which can aid identification by providing an additional Cys sequence constraint, and provides a significant reduction in complexity of the polypeptide mixture to be analyzed.



To demonstrate the ICAT strategy, a protein extract from cul-

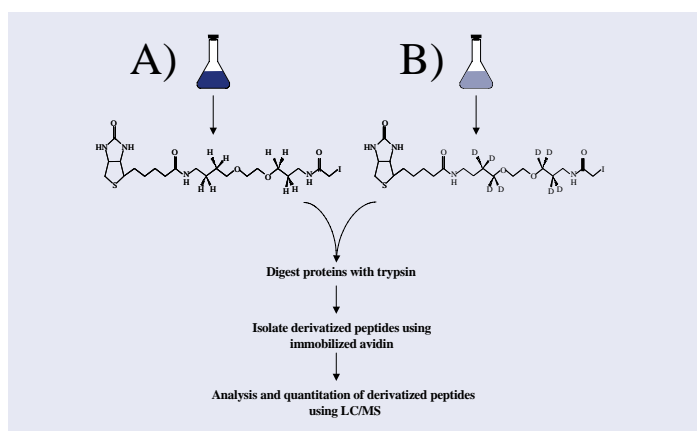


Figure 2

Schematic representation of the isotope-coded affinity tag (ICAT) strategy. Proteins are separately extracted from cells grown under two different conditions (A and B). The proteins for each sample are labeled either with the light (ICAT- $\text{D}^0$ ) or heavy (ICAT- $\text{D}^8$ ) ICAT reagent. After labeling the proteins are pooled and digested with trypsin. The modified peptides are isolated by affinity chromatography and analyzed by capillary LC/MS.

tured mouse B16 melanoma cells was divided into two equal aliquots. One aliquot was derivatized with the light isotopic version of the ICAT- $\text{D}^0$  reagent and the other with the ICAT- $\text{D}^8$  reagent. The derivatized proteomes were pooled, digested with trypsin, and the labeled Cys-polypeptides isolated using avidin affinity chromatography. The peptide mixture was analyzed in a single capillary LC-MS experiment by FTICR. In this single analysis, hundreds of pairs of Cys-polypeptides with the expected integral mass difference of 8.0 Da were observed. A few of these peptides are shown in Figure 3. The average ratio of peak areas for the distinct isotopically labeled versions of each peptide was  $\sim 1.01$ . Since identical aliquots of the proteome sample were used in this experiment, average ratio of peak areas for the distinct isotopically labeled versions of each peptide was  $\sim 1.01$ , consistent with the expected results.

Recently, an alternative strategy has been developed that combines  $^{15}\text{N}$ -metabolic labeling and post-extraction cysteine affini-



# New Products

CATALOG#	COMPOUND (Isotope, Atom % Enrichment)	SIZE	PRICE
CNLM-3726	Acetaminophen (acetyl- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 98%+)	1g	\$ 990
CLM-813	Acrylamide (1,2,3- <sup>13</sup> C <sub>3</sub> , 99%)	0.1g	\$ 675
DLM-821	Acrylamide (2,3,3-D <sub>3</sub> , 98%)	1g	\$ 450
CLM-4723	Adipic Acid ( <sup>13</sup> C <sub>6</sub> , 99%)	0.5g	\$ 700
CLM-3589	L-Alanine-N- <i>t</i> -BOC (U- <sup>13</sup> C <sub>3</sub> , 98%)	0.25g	\$ 725
CNLM-4355	L-Alanine-N-FMOC (U- <sup>13</sup> C <sub>3</sub> , 98%; <sup>15</sup> N, 98%)	0.1g 0.25g	\$ 350 \$ 700
CNLM-4805	Aniline ( <sup>13</sup> C <sub>6</sub> , 98%+; <sup>15</sup> N, 98%+)		Request Price
NLM-1264	L-Arginine-N-FMOC, PMC (+isopropyl ether) (U- <sup>15</sup> N <sub>4</sub> , 98%)	0.1g	\$ 1500
CNLM-4226	L-Arginine-N-FMOC, PMC (+isopropyl ether) (U- <sup>13</sup> C <sub>6</sub> , 98%; U- <sup>15</sup> N <sub>4</sub> , 98%) (contains <5% D-isomer)	0.1g	\$ 2900
CNLM-4752	L-Aspartic Acid-N-FMOC, Beta-O- <i>t</i> -Butyl Ester ( <sup>13</sup> C <sub>4</sub> , 98%; <sup>15</sup> N, 98%)		Request Price
DLM-4528	Bis-Tris (D <sub>19</sub> , 98%)	0.5g 1g	\$ 420 \$ 650
DLM-4862	Cacodylic Acid (D <sub>7</sub> , 98%)	0.5g	\$ 500
CLM-4899	L-Citrulline (ureido- <sup>13</sup> C, 99%)	1g	\$ 1600
DLM-4532	L-Cysteine, S-trityl (3,3-D <sub>2</sub> , 98%)	0.1g	\$ 310
DLM-4721	L-Cysteine-N-FMOC, S-trityl (3,3-D <sub>2</sub> , 98%)	0.1g 0.5g	\$ 450 \$ 1350
CNLM-4722	L-Cysteine-N-FMOC, S-trityl (U- <sup>13</sup> C <sub>3</sub> , 98%; <sup>15</sup> N, 98%)	0.1g	\$ 1200
CNLM-4510	5,6-Dihydrouracil ( <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 98%+)	25mg	\$ 1200
CLM-3672	Erythromycin (N,N-dimethyl- <sup>13</sup> C <sub>2</sub> , 90%)(90-95% A isomer)	1g	\$ 800
CLM-3758	Erythromycin Lactobionate Salt (N,N-dimethyl- <sup>13</sup> C <sub>2</sub> , 90-95%)	1g	\$ 990
CLM-4326	Fluorobenzene ( <sup>13</sup> C <sub>6</sub> , 99%)	0.1g	\$ 350
CDLM-4599	Formaldehyde ( <sup>13</sup> C, 99%; D <sub>2</sub> , 98%) Approx 20% w/w in D <sub>2</sub> O	1ml	\$ 399
CLM-3639	Glycine-N-FMOC (1- <sup>13</sup> C, 99%)	1g	\$ 525
CNLM-4357	Glycine-N-FMOC (U- <sup>13</sup> C <sub>2</sub> , 98%; <sup>15</sup> N, 98%)	0.25g	\$ 700
CNLM-4393	5-Hydroxyuracil ( <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N, 98%)	5mg	\$ 895
CDLM-4611	α-Ketobutyric Acid, Sodium salt ( <sup>13</sup> C <sub>4</sub> , 98%; 3,3-D <sub>2</sub> , 98%) (contains 5% Dimer)	0.1g	\$ 815
DLM-4214	α-Ketoisocaproic Acid, Sodium salt (isopropyl-D <sub>7</sub> , 98%)	0.1g	\$ 590
CDLM-4418	α-Ketoisovaleric Acid, Sodium salt (U- <sup>13</sup> C <sub>5</sub> , 97-98%; 3-D <sub>1</sub> , 98%)	0.25g	\$ 990
CNLM-4345	L-Leucine-N-FMOC (U- <sup>13</sup> C <sub>6</sub> , 98%; <sup>15</sup> N, 98%)	0.1g	\$ 450
CNLM-4754	L-Lysine-α-N-FMOC, ε-N- <i>t</i> -BOC ( <sup>13</sup> C <sub>6</sub> , 98%; <sup>15</sup> N <sub>2</sub> , 98%)		Request Price
CNLM-4358	L-Methionine-N-FMOC (U- <sup>13</sup> C <sub>5</sub> , 98%; <sup>15</sup> N, 98%)		Request Price
CLM-4477	Oleic Acid, Potassium Salt (1- <sup>13</sup> C, 99%)	1g	\$ 575
DLM-4261	L-Ornithine•2HCl (5,5-D <sub>2</sub> , 98%)	0.25g	\$ 675
NLM-3610	L-Ornithine•HCl ( <sup>15</sup> N <sub>2</sub> , 98%)	0.25g	\$ 520
CNLM-4362	L-Phenylalanine-N-FMOC (U- <sup>13</sup> C <sub>9</sub> , 98%; <sup>15</sup> N, 98%)	0.1g	\$ 450
DLM-4781	PIPES (Piperazine-N,N'-bis (2-ethanesulfonic acid)) (D <sub>16</sub> , 99%+; OD, 70%)	0.25g	\$ 375
CLM-3943	Potassium Palmitate (U- <sup>13</sup> C <sub>16</sub> , 98%)	0.5g	\$ 975
CNLM-4347	L-Proline-N-FMOC (U- <sup>13</sup> C <sub>5</sub> , 98%; <sup>15</sup> N, 98%)	0.1g	\$ 450
CNLM-4755	L-Serine-N-FMOC, O- <i>t</i> -Butyl Ether (U- <sup>13</sup> C <sub>3</sub> , 98%; <sup>15</sup> N, 98%) (contains 3% D-isomer)		Request Price
CLM-3980	Sodium Octanoate (2,4,6,8- <sup>13</sup> C <sub>4</sub> , 99%)	0.25g	\$ 900
NLM-3995	Thymine (1,3- <sup>15</sup> N <sub>2</sub> , 98%)	0.1g	\$ 450
CNLM-4349	L-Tyrosine-N-FMOC, O- <i>t</i> -Buyl Ether (U- <sup>13</sup> C <sub>9</sub> , 98%; <sup>15</sup> N, 98%)	0.1g	\$ 990
CNLM-4348	L-Valine-N-FMOC (U- <sup>13</sup> C <sub>5</sub> , 98%; <sup>15</sup> N, 98%)	0.1g 0.5g	\$ 450 \$ 800

# NEW WEB SITE

## [www.isotope.com](http://www.isotope.com)

CIL's new Web site was launched in December. The new Web site has a shopping cart feature to process orders and quote requests as well as a searchable database of CIL products. All products are organized by product group and application category to assist you in finding the right product for your application. In addition to being database driven, this site will have a completely new look and feel. We will continually add new products and special promotions to the Web site. Add our site to your list of favorites so that you don't miss out on savings opportunities.



## Email Promotions

If you would like to receive email notifications of CIL's special offers and new products, please send your email address to [cilsales@isotope.com](mailto:cilsales@isotope.com). Some promotions are only being announced on our Web site and through email notifications. We'd be happy to include you in these mailings.



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### *International*

From left to right: Katherine Belisle *International Accounts Manager*, Cindy Paszko, Michael Robey, Tracy MacDonald.

## SPECIAL OFFER

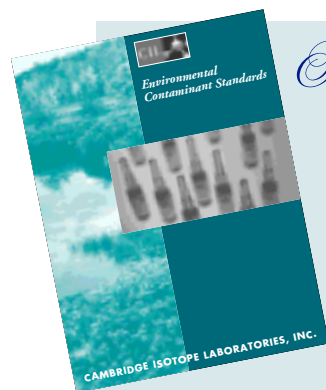
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# available Literature from Cambridge Isotope Laboratories



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- An **index**, containing both individual compounds, and components of formulations, to make it easier to locate products.
- Compounds that logically belong in more than one section are listed in **multiple locations**. For instance, a Pesticide that is also a suspected Endocrine Disrupting Compound (EDC) is listed in both the Pesticide **and** the EDC section.
- New products are highlighted with a *New* notation for fast identification.

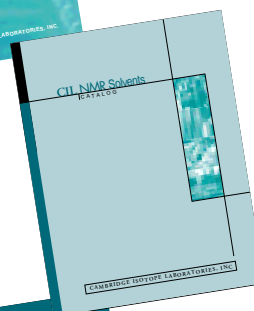
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- Cerilliant (our long-term collaborator, formerly known to the analytical community as Radian) has improved specification of the 17 "Toxic" Dioxins and Furans to  $\pm 5\%$  as well. ISO 9001 certification was renewed by Cerilliant during their most recent audit.
- Dozens of new Endocrine Disrupting Compounds (EDCs), for a total of over 70 suspected EDCs.
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