Isotope Labeled Peptides

Targeted Quantification of Protein and Post-translational Modifications in Complex Protein Mixtures

FEBRUARY 1, 2022

Genomics research shows that more than a million proteins are encoded by approximately 30,000 human genes. Proteomics, the study of proteins encoded by the genome, includes identification of post-translational modifications, structural analyses, protein localization studies, and protein quantitation. Mass spectroscopy-based techniques have evolved as a powerful tool in proteomics. Stable isotope-labeled peptides (SIL peptides) are chemically and physically indistinguishable from their endogenous counterparts with respect to retention time, ionization efficiency, and fragmentation pathways. Therefore, either are ideal internal standards and template analytes. Peptides can be labeled with one or more isotopes of hydrogen, carbon, nitrogen, or oxygen by incorporating amino acids containing the desired isotopes such as deuterium (D), 13C, 15N, or 18O into the peptide during synthesis.

Methodology

The Absolute Quantification method (AQUA) enables targeted quantification of protein and post-translational modifications in complex protein mixtures using SIL peptides as internal standards. The SIL peptide is introduced into a biological sample during or after protease digestion. The heavy SIL peptide and its light endogenous peptide fragment are detected by selected reaction monitoring (SRM) in a mass spectrometer. Based on the known amount of SIL peptide added, and the intensity ratio of both peptides, the amount of endogenous protein can be calculated.

SIL Peptides in Structural Analysis

Nuclear Magnetic Resonance (NMR) is a powerful technique for investigating structural information, dynamics, and molecular interactions of biomolecules. This technique can be used to measure relaxation rates of biomolecules as they dissociate from their bound target. Peptides labeled with D (spin of 1), ^{13}C (spin 1/2), and ^{15}N (spin 1/2) are suitable for NMR studies of proteins. A combination of NMR spectroscopy and segmental isotopic labeling is used to study the mechanism of protein splicing (e.g., the structure of an active protein splicing precursor), a post-translational autocatalytic process in which an intervening sequence, termed an intein, is removed from a host protein, the extein.

Isotopic Amino Acid Table

Amino Acid	Isotope	Mass Differnce	Isotopic Enrichment
Alanine	$^{13}C_3$, ^{15}N	4	>99%
Arginine	$^{13}C_6$, $^{15}N_4$	10	>99%
Isoleucine	$^{13}C_6$, ^{15}N	7	>99%
Leucine	$^{13}C_6$, ^{15}N	7	>99%
Lysine	$^{13}C_6$, $^{15}N_2$	8	>99%
Phenylalanine	$^{13}C_9$, ^{15}N	10	>99%
Proline	$^{13}C_5$, ^{15}N	6	>99%
Valine	$^{13}C_5$, ^{15}N	6	>99%

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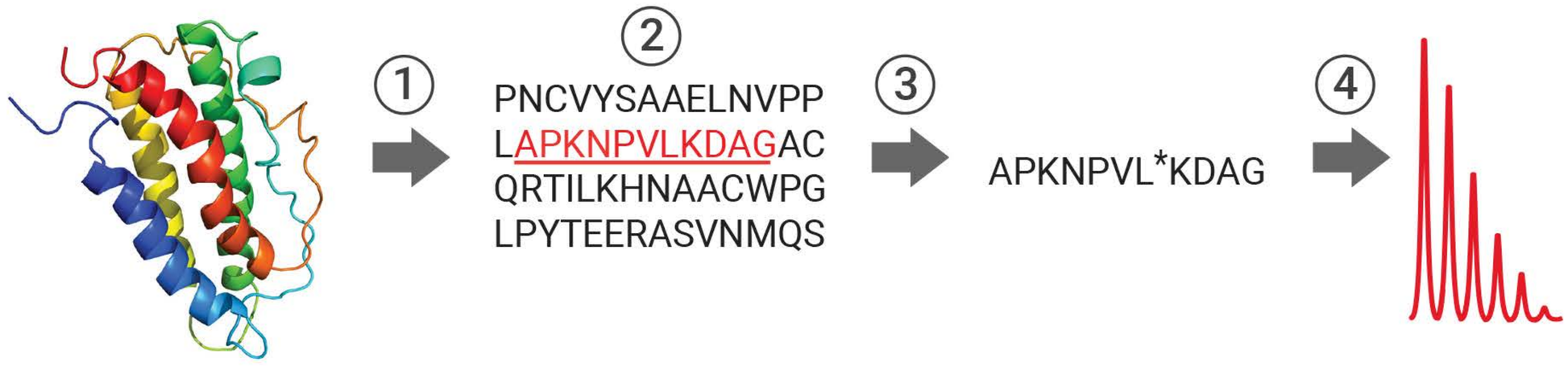
Isotopic Labeling Chemistry



Stages of Absolute Quantification

1. Proteins of interest identified/selected
2. Peptide internal standard sequence selected
3. Synthesis of SIL peptide
4. Detailed compositional analysis of SIL peptide by LC-MS/MS and method optimization
5. SIL peptide added to protein prior to enzymatic digestion
6. Fragmented peptides are isolated
7. AQUA using mass spectrometric analysis

High Quality SIL Peptides from CPC

Absolute quantitation of a complex protein mixture at very low concentrations and structural studies requires high-quality peptides enriched with stable isotopes. The CPC Scientific SIL Peptide Custom Synthesis Service guarantees superior quality and high isotopic enrichment. These stable isotopic peptides are synthesized using the latest Fmoc solid-phase peptide technology in our state-of-the-art peptide laboratory. All heavy isotope-labeled peptides undergo mass spectrometric analysis and stringent analytical HPLC to establish the final purity and assure that our customers receive only the highest quality peptides for absolute quantitation and other studies.

Two major isotopes, ^{15}N and ^{13}C, are incorporated into a specific amino acid sequence of peptides. Each atom contains over 99% of an enriched isotope and can be located at multiple positions within the peptide. For example, a Leu amino acid containing one ^{15}N and six ^{13}C, and a peptide containing one such isotope-labeled Leu amino acid, has seven units of molecular weight higher than the corresponding unlabeled peptide.

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SIL Peptide Applications

- Functional quantitative proteomics
- Quantitation of post translationally-modified proteins
- Protein structure analysis
- Protein expression monitoring
- Biomarker discovery
- Pharmacokinetics
- Metabolomics
- Clinical biochemistry for drug and metabolite monitoring
- Anti-doping testing
- Cell signal profiling and pathway validation
- Protein cross-linking analysis

SIL Peptide Modifications

- Phospho-Tyr, Ser, Thr (single or multiple)
- Sulfo-Tyr (single or multiple)
- Methylated Arg, Lys
- Chloro-Tyr
- Met-oxidized
- Pyroglutamic acid

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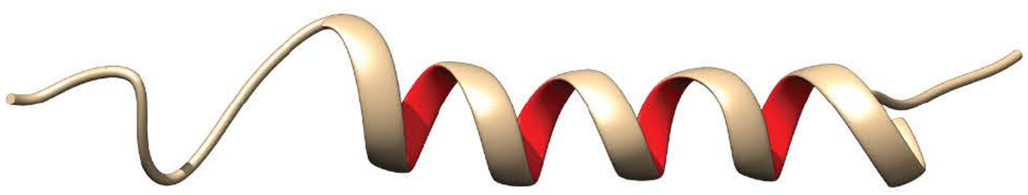
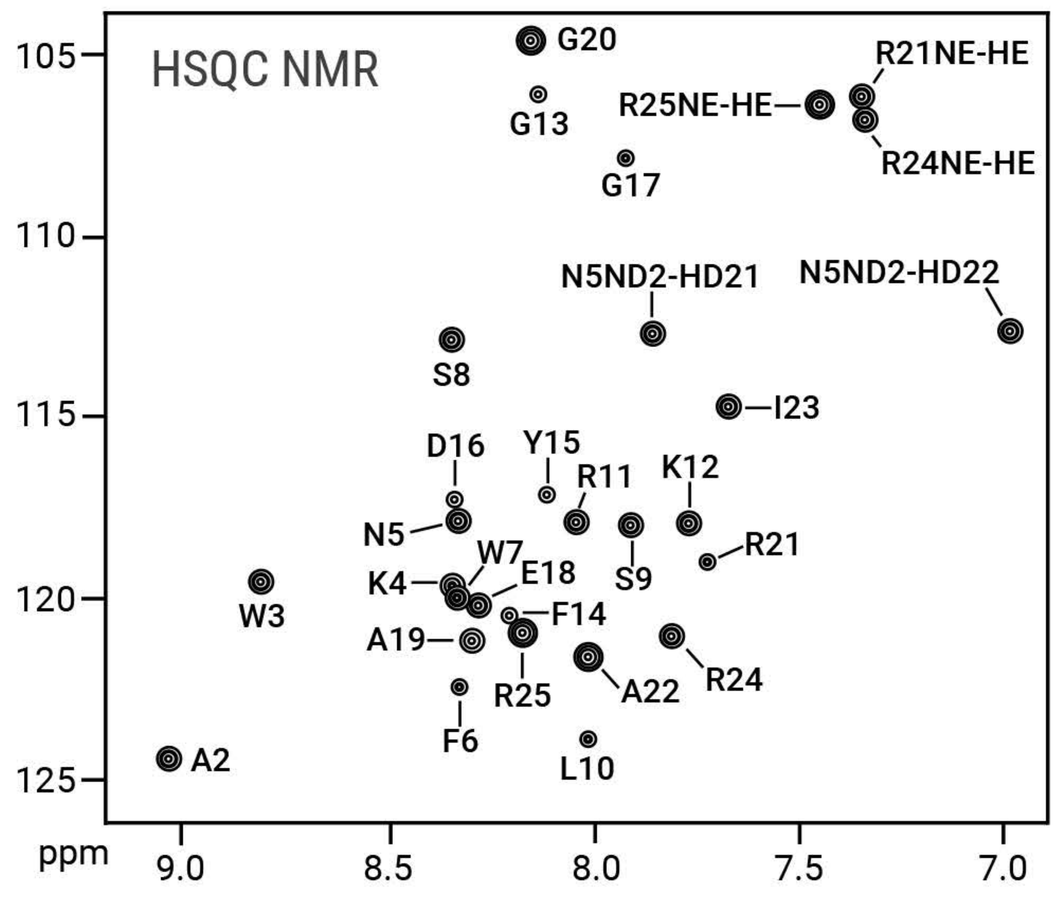
HSQC NMR Techniques

Heteronuclear single quantum coherence (HSQC) NMR provides well resolved spectra for structural elucidation. HSQC of Plantaricin shows that the peptide adopts a well-defined alpha-helical structure in TFE, an environment that resembles cellular membranes.



Plantaricin Peptide



Rogne, Per, et al. “Three-dimensional structure of the two-peptide bacteriocin plantaricin JK.” *Peptides* 30.9 (**2009**): 1613-1621. (Figure adapted from HSQC data in reference)

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Isotope-Labeled Peptide Citations

Synthetic Hcrt1 with13Cand15N stabile isotopemodification on two leucine amino acids was used for internalstandard (ISTD) calibration; Glp-P-L(U13C6,15N)PDCCR-QKTCSCR-L(U13C6,15N) YELLHGAGNHAAGILTL-NH2(CPC Scientific, Sunnyvale, CA)

Bårdsen, Kjetil, Michaela D. Gjerstad, Markku Partinen, Ingeborg Kvivik, Anne Bolette Tjensvoll, Peter Ruoff, Roald Omdal, and Cato Brede. "Considerably lower levels of hypocretin-1 in cerebrospinal fluid is revealed by a novel mass spectrometry method compared with standard radioimmunoassay. " *Analytical Chemistry* 91, no. 14 (**2019**): 9323-9329.

"Stable isotope-labelled (SIL) peptides were synthesized by CPC Scientific. Leucine residues were selectively labeled using 13C/15N-labelled amino acids resulting in the addition of 7 Da to the peptide mass (Supplementary Table S1). The reported purity of each synthesized peptide was more than 95% based on HPLC analysis. In addition, amino acid analysis (AAA) was performed to determine the peptide content for each standard and these values were used to establish concentrations used in quantitative studies."

Zhen, Eugene Y., et al. "Circulating FGF21 Proteolytic Processing Mediated by Fibroblast Activation Protein." *Biochemical Journal* (**2015**): BJ20151085.

"antipeptide (EGVYVHPV), angiotensin II, human (DRVYIHPF), and isotopically (13C) labeled heptapeptide (AAAAHAA-NH2 [where "A" indicates a carbon thirteen (13C) isotope on the alanine carbonyl group]) were purchased from CPC Scientific Incorporated (Sunnyvale, CA)"

Harper, Brett, Mahsan Miladi, and Touradj Solouki. "Loss of internal backbone carbonyls: additional evidence for sequence-scrambling in collision-induced dissociation of y-type ions." *Journal of The American Society for Mass Spectrometry* 25.10 (**2014**): 1716-1729.

"Both the stable isotope-labeled (SIL) and unlabeled apelin peptides [apelin-36, apelin-17, apelin-13, (pyr) apelin-13, and apelin-12] were synthesized by CPC Scientific (Sunnyvale, CA, USA)."

Zhen, Eugene Y., Richard E. Higgs, and Jesus A. Gutierrez. "Pyroglutamyl apelin-13 identified as the major apelin isoform in human plasma." *Analytical Biochemistry* 442.1 (**2013**): 1-9.

"The internal standard (ISTD) WIL*GDVFIREYYSV*FDR was ordered from CPC Scientific (Sunnyvale, Calif., USA) and contained L*6×13C 1×15N and V*5×13C 1×15N. The ISTD contained an internal trypsin cleavage site in order to monitor the digestion with trypsin to completion."

Dekker, Petrus Jacobus Theodorus, René Marcel De Jong, and Cornelis Marinus Muijlwijk. "Chymosine enzyme variants." *U.S. Patent Application* No. 14/397,866.

"The OXM peptides 1–37, 3–37 and 4–37 as well as their corresponding stable isotope-labeled internal standards (each containing one [13 C 9 , 15 N]-labeled phenylalanine and two [13 C 6 , 15 N]-labeled leucine residues) were synthesized by CPC Scientific, Inc."

Cox, Jennifer M., et al. "Characterization and quantification of oxyntomodulin in human and rat plasma using high-resolution accurate mass LC-MS." *Bioanalysis* 8.15 (**2016**): 1579-1595.

"Glucagon synthetic peptide and heavy isotope–labeled internal standards (ISs) of oxyntomodulin and glucagon containing 2 [13 C 6 – 15 N]-labeled leucines and 1 [13 C 9 – 15 N]-labeled phenylalanine were synthesized by CPC Scientific.."

Lee, Anita YH, et al. "Multiplexed quantification of proglucagon-derived peptides by immunoaffinity enrichment and tandem mass spectrometry after a meal tolerance test." *Clinical Chemistry* 62.1 (**2016**): 227-235.

"Two peptides of the polyserine tract of AmVg and NvVg were synthesized by CPC Scientific (Sunnyvale, CA, USA) for NMR analysis. The synthesis was performed after isotope-labeling expression attempts in Escherichia coli were deemed unsuitable for the project because of the very low final yield. [..] The peptides had the following sequences: AmVg (residues 358–392): EKLKQDILNLRTDISTS(Sp)SS(15I) SSSEENDFWQPKPT AmVg (residues 336–385): R(15V) SKT(15A)MNSNQI(15V)SDNS(15L)-(15S)STEEK(15L) KQDI(15L)N(15L)RTDI(15S)S(15S)(Sp)S(15A)IS (15S)(15S) EEND. NvVg (residues 351–385): EHKHSDESTSE(Sp)FES(15I) ADNNDDSYFQRKPKLTEAP." NvVg (residues 335–372): RPNK(15L)N(15L)QRRHDHKS(15G)EHKHSDE(15S)S-(15S) E(Sp)FE(15A)I(15A)DNNDD.

Havukainen, Heli, et al. "A vitellogenin polyserine cleavage site: highly disordered conformation protected from proteolysis by phosphorylation." *The Journal of Experimental Biology* 215.11 (**2012**): 1837-1846.

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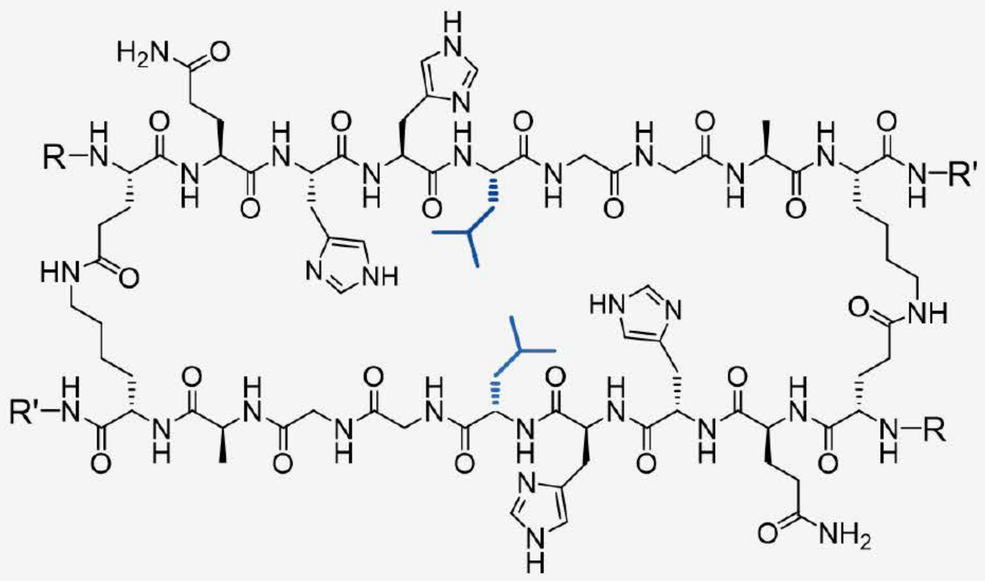

R = H-Arg-Leu-Thr-Ile-Gly-Glu-Gly-
R' = -Gln-Ala-Gly-Asp-Val-OH

"Peptide Stable isotope–labeled internal standard peptide (Q 7 and K 15 reciprocally cross-linked dimer of LTIGEGQQHHL*GGAKQAGDV, where L* represents 13 C 6 , 15 N 1 -labeled leucine) was synthesized and analyzed for purity and amino acid content (CPC Scientific)"

Wang, Weixun, et al. "Quantification of circulating D-dimer by peptide immunoaffinity enrichment and tandem mass spectrometry." *Analytical Chemistry* 84.15 (**2012**): 6891-6898.

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