Product Information

U-15N-SILAM-Mouse Diet
U-14N-SILAM-Mouse Diet

A mouse feed which is composed of a conventional protein-free diet, supplemented with a ^{15}N- or unlabeled *Ralstonia eutropha*-based protein hydrolysate.

Background

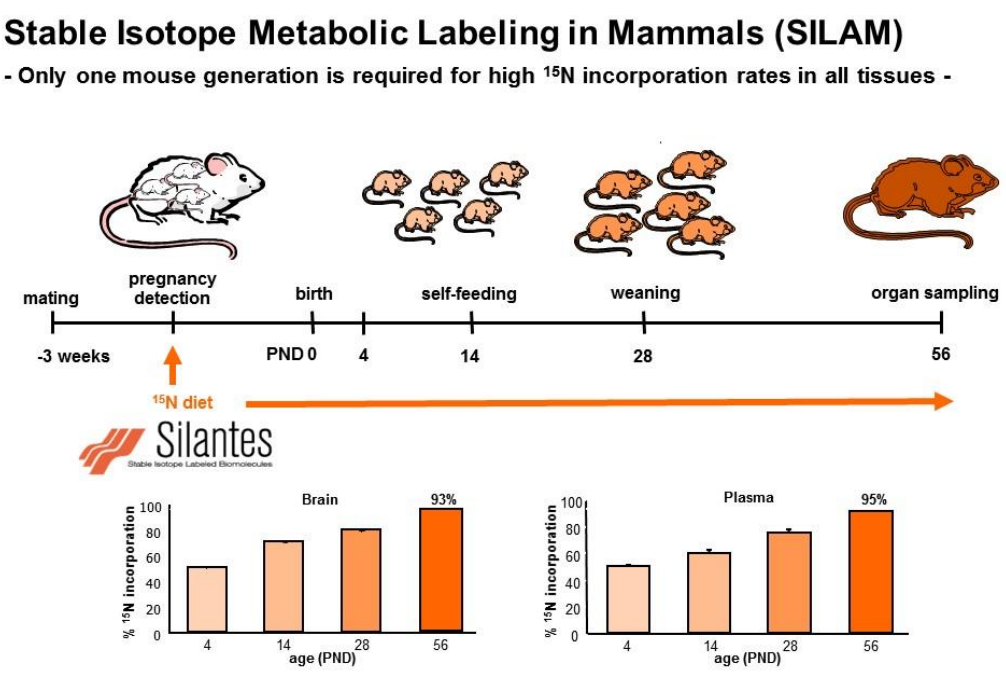
Stable isotope labeling is the most reliable and accurate method for quantitative proteomics and metabolomics.

In cooperation with Prof. Chris Turck's group at the *Max Planck Institute of Psychiatry Silantes* has developed a ^{15}N-diet specifically designed for mouse uniform metabolic labeling. The diet is made of a *Ralstonia eutropha* bacteria hydrolysate as ^{15}N protein source. When labeling is started *in utero* and offspring continued to be fed for two months, only one mouse generation is needed to achieve greater than 90% ^{15}N incorporation rates in all tissues (Fig. 1) (*Frank E, et al. 2009*).

Almost every nitrogen atom in the organism is present as ^{15}N isotope. This includes proteins, metabolites and DNA/RNA.

Tissue including adrenal gland, brain sections, heart, kidney, liver, lung, muscle, pancreas, plasma, red blood cells, spleen and thymus has been retrieved, snap frozen in liquid nitrogen, stored at -80°C and is available upon request.

Figure 1

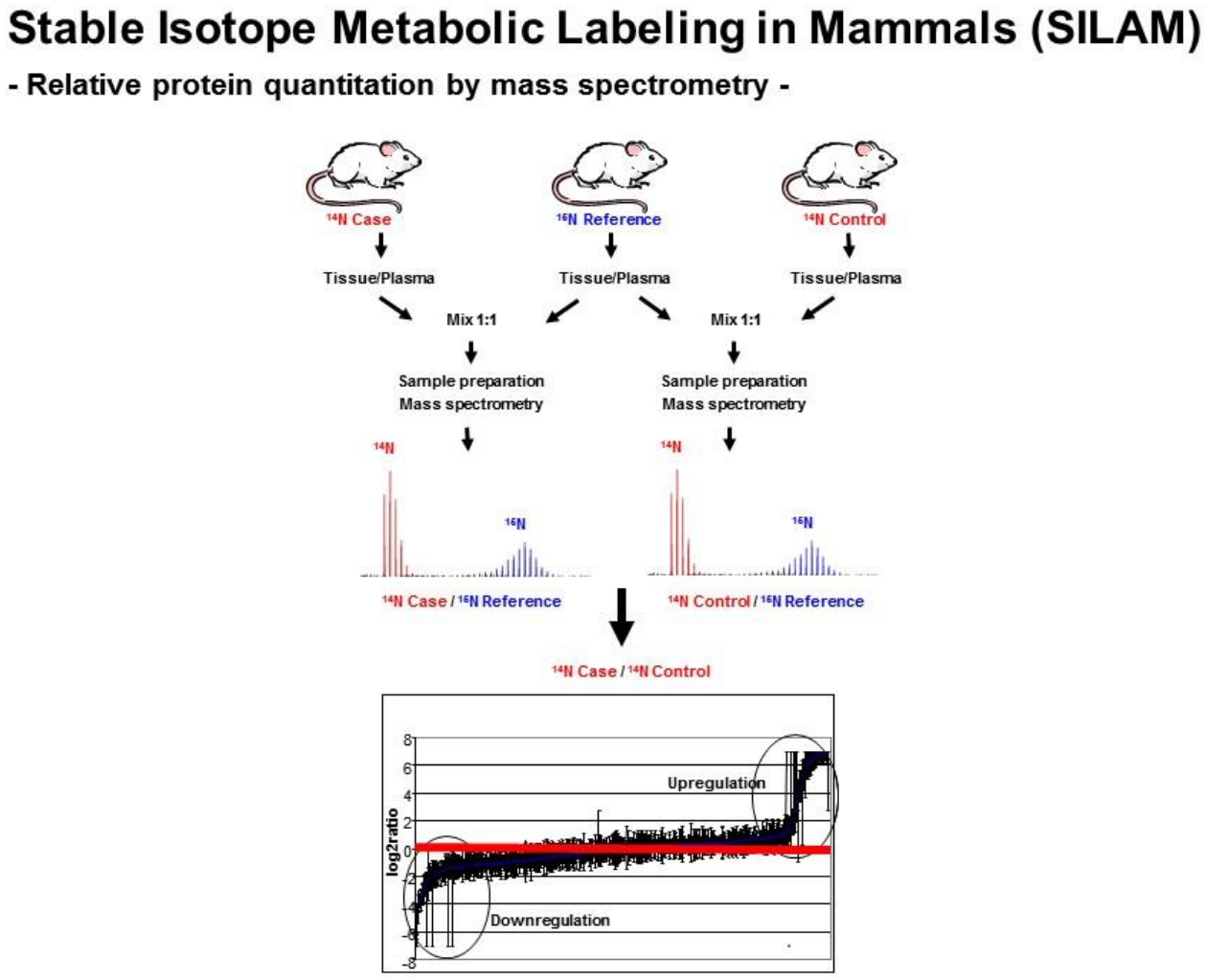


Applications

Proteomics – relative protein quantification:

- Completely labeled mouse specimens as reference material for relative protein quantification by mass spectrometry (Fig. 2) (*Wu CC, et al. 2004*; *Filiou MD, et al. 2011*; *Zhang Y, et al. 2011*). The labeled tissue specimens (adrenal gland, brain sections, heart, kidney, liver, lung, muscle, pancreas, plasma, red blood cells, spleen, thymus) can be used for comparing two or more states of the same type of tissue from the same species.

Figure 2



Proteomics – protein turnover:

- Partially labeled mouse specimens for global and targeted protein turnover analysis by mass spectrometry (Figs. 3 and 4) (*Zhang YY, et al. 2011*; *Ko HG et al. 2018*). *ProTurnyzer* software for the analysis of individual protein turnover by mass spectrometry developed in Prof. Turck's laboratory is available upon request.

Figure 3

Global and Targeted Protein Turnover Analysis in Live Rodents
- *partial* stable isotope metabolic labeling -

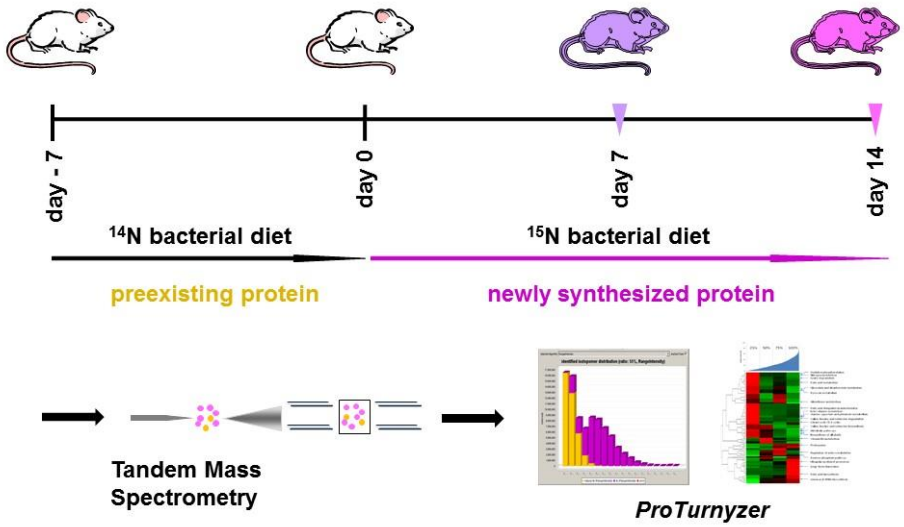
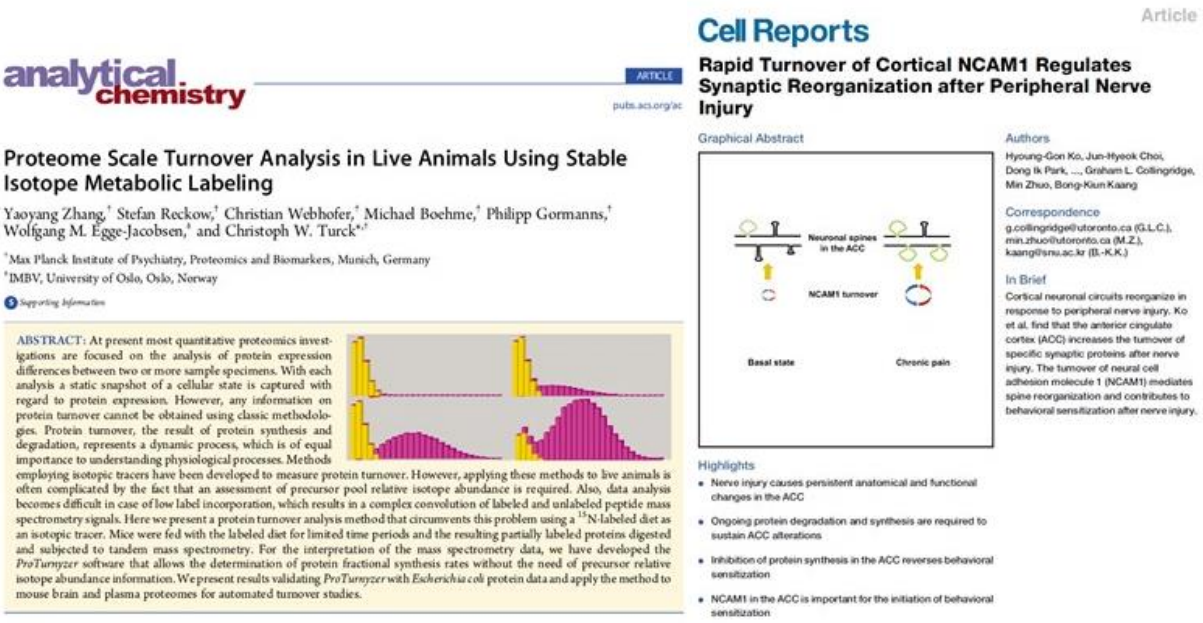


Figure 4

Global and Targeted Protein Turnover Analysis in Live Rodents
- *partial* stable isotope metabolic labeling -



analytical chemistry

ARTICLE

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Proteome Scale Turnover Analysis in Live Animals Using Stable Isotope Metabolic Labeling

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Supporting Information

ABSTRACT: At present most quantitative proteomics investigations are focused on the analysis of protein expression differences between two or more sample specimens. With each analysis a static snapshot of a cellular state is captured with regard to protein expression. However, any information on protein turnover cannot be obtained using classic methodologies. Protein turnover, the result of protein synthesis and degradation, represents a dynamic process, which is of equal importance to understanding physiological processes. Methods employing isotopic tracers have been developed to measure protein turnover. However, applying these methods to live animals is often complicated by the fact that an assessment of precursor pool relative isotope abundance is required. Also, data analysis becomes difficult in case of low label incorporation, which results in a complex convolution of labeled and unlabeled peptide mass spectrometry signals. Here we present a protein turnover analysis method that circumvents this problem using a ^{15}N-labeled diet as an isotopic tracer. Mice were fed with the labeled diet for limited time periods and the resulting partially labeled proteins digested and subjected to tandem mass spectrometry. For the interpretation of the mass spectrometry data, we have developed the *ProTurnyzer* software that allows the determination of protein fractional synthesis rates without the need of precursor relative isotope abundance information. We present results validating *ProTurnyzer* with *Escherichia coli* protein data and apply the method to mouse brain and plasma proteomes for automated turnover studies.

Cell Reports

Article

Rapid Turnover of Cortical NCAM1 Regulates Synaptic Reorganization after Peripheral Nerve Injury

Graphical Abstract

Neuronal spines in the ACC
NCAM1 turnover
Basal state
Chronic pain

Authors

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In Brief

Cortical neuronal circuits reorganize in response to peripheral nerve injury. Ko et al. find that the anterior cingulate cortex (ACC) increases the turnover of specific synaptic proteins after nerve injury. The turnover of neural cell adhesion molecule 1 (NCAM1) mediates spine reorganization and contributes to behavioral sensitization after nerve injury.

Highlights

- Nerve injury causes persistent anatomical and functional changes in the ACC
- Ongoing protein degradation and synthesis are required to sustain ACC alterations
- Inhibition of protein synthesis in the ACC reverses behavioral sensitization
- NCAM1 in the ACC is important for the initiation of behavioral sensitization

Metabolomics – relative metabolite quantification and structure validation:

Labeled metabolite standards from different tissues (adrenal gland, brain sections, heart, kidney, liver, lung, muscle, pancreas, plasma, red blood cells, spleen, thymus).

- Biomarker discovery and verification analogous to proteomics (see above).

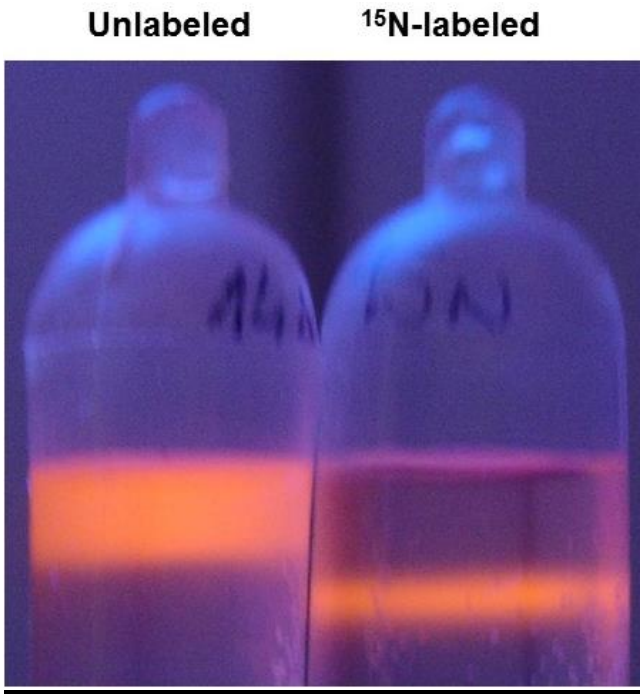
- Molecular structure confirmation.

Genomics:

- Utility not yet explored.

Figure 5

Density gradient centrifugation of DNA isolated from unlabeled and completely ^{15}N-labeled mouse brain tissue.



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