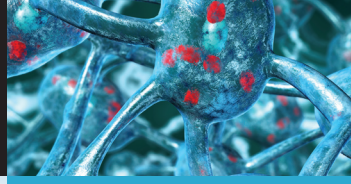



Investigator Spotlight

# Protein Turnover

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For an organism to respond to changes in its environment, the abundance of specific proteins must be altered. How rapidly this change can be brought about is controlled in part by the rate of turnover of the proteins; a protein that has a high rate of turnover can be increased in abundance, or removed from the protein pool, very rapidly. To determine the rate of protein synthesis using mass spectrometry, the incorporation of a stable isotope-labeled tracer into newly synthesized proteins can be monitored. Similarly, to assess rates of degradation, loss of the tracer is followed. One of the classes of tracer used to monitor flux through the protein pool is stable isotope-labeled amino acids.[1,2]

The Protein Function Group has always had a keen interest in proteome dynamics, initially in simple cellular systems, such as yeast and mammalian cell culture but also in more complex whole animal models. In one of the first studies of proteome dynamics, turnover rates of yeast proteins from cells grown in glucose-limiting conditions in a chemostat were determined using L-Leucine ($D_{10}$, 98%) (DLM-567) in the growth media[3,4] in an "unlabeling" experiment.[2] In a similar "dynamic-SILAC" study, human A549 adenocarcinoma cells were labeled with L-Arginine•HCl ($^{13}C_6$, 99%) (CLM-2265-H) and the rate of loss of label monitored for almost 600 intracellular proteins using a geLC/MS approach.[5]

In animal systems, the challenge becomes one of effective administration of the stable isotope tracer. We have taken a simple approach and administer label via incorporation into the diet. In a study of domestic fowl, a semi-synthetic diet was formulated for chickens, containing L-Valine ($D_8$, 98%) (DLM-488) at a relative isotopic abundance (RIA) of 0.5 (in other words, half of the valine was isotopically labeled). This might be seen as a compromise, but totally synthetic diets can be unpalatable and so partial labeling allowed design of a diet to sustain the high growth rate of the young chicks.[6] Proteins from skeletal muscle were assessed over the five-day labeling period and turnover rates were determined, after first calculating the RIA of the muscle precursor pool using multiply labeled peptides based on mass isotopomer distribution analysis.[7] For mice, we have used a semi-synthetic diet containing L-Valine ($D_8$, 98%) in which we simply added the stable isotope-labeled amino acid to the same level as that present in a standard laboratory chow. The rate of turnover of proteins in different organs (liver, kidneys, heart and skeletal muscle) was assessed – using major urinary proteins (MUPs) synthesised in the liver and excreted in the urine – to track the labeling trajectory non-invasively without the requirement for large numbers of animals.[8] The same diet has also been used in a different study, to track the baseline replacement of reproductive proteins from the epididymis and seminal vesicles of mice, to predict which proteins are mostly likely to respond under conditions of sperm competition.[9] As a further part of this study, a semi-synthetic diet containing L-Lysine•2HCl ($^{13}C_6$, 99%) (CLM-2247-H) at an RIA of 0.5 will be manufactured to assess the relative investment of different males mating under different levels of sperm competition.

The use of stable isotope-labeled amino acids enables the turnover trajectories of individual proteins in a diverse range of samples to be determined with relative ease, especially with the increasing software options for downstream data-analysis. We have shown that simple supplementation of a standard laboratory diet with an amino acid, at an RIA less than 1, is a cost-effective and biologically defendable approach to whole animal studies.

### References

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## Related Products

| Catalog No. | Description |
|---|---|
| CLM-2265-H | L-Arginine•HCl ($^{13}C_6$, 99%) |
| DLM-567 | L-Leucine ($D_{10}$, 98%) |
| CLM-2247-H | L-Lysine•2HCl ($^{13}C_6$, 99%) |
| DLM-488 | L-Valine ($D_8$, 98%) |