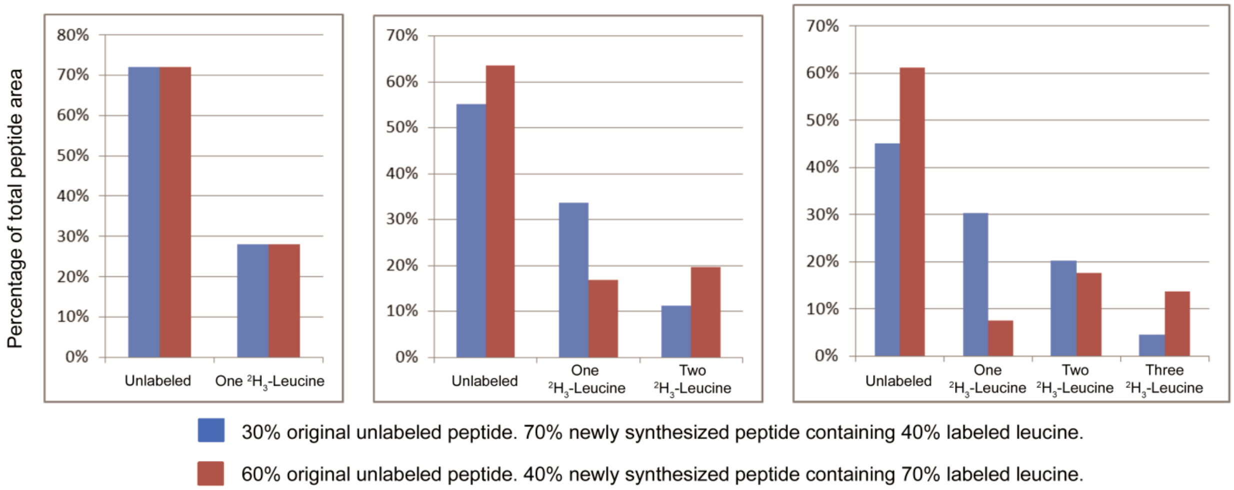
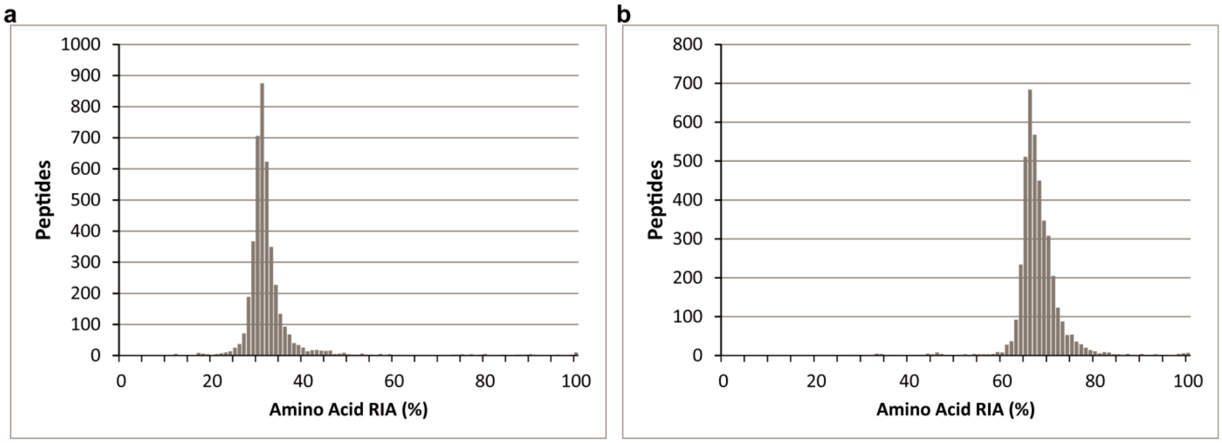
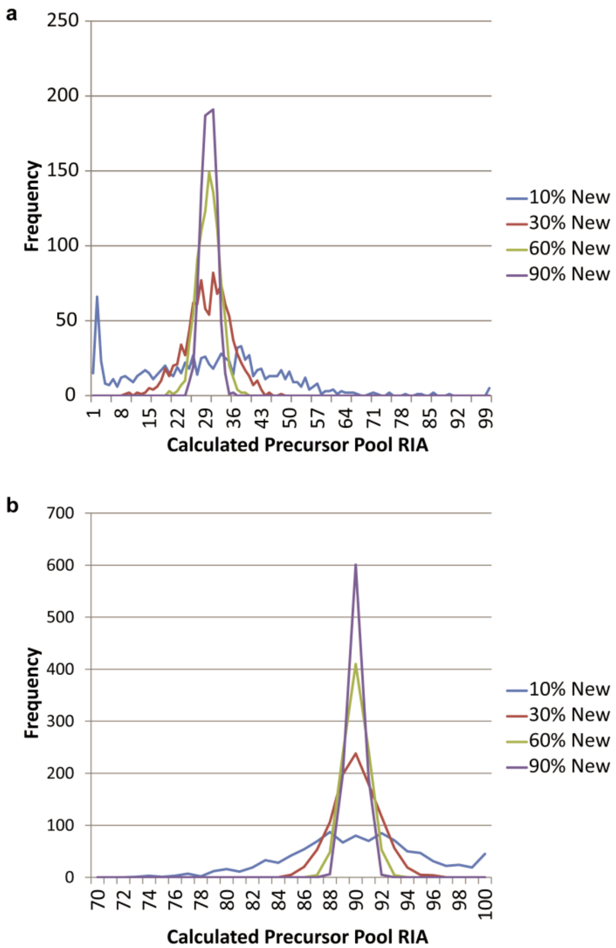
Supplementary Materials



Supplementary Figure 1 – Peptides with multiple leucine residues (center and right panels) can be used to distinguish between labeling scenarios that are indistinguishable for peptides with a single leucine residue (left panel).

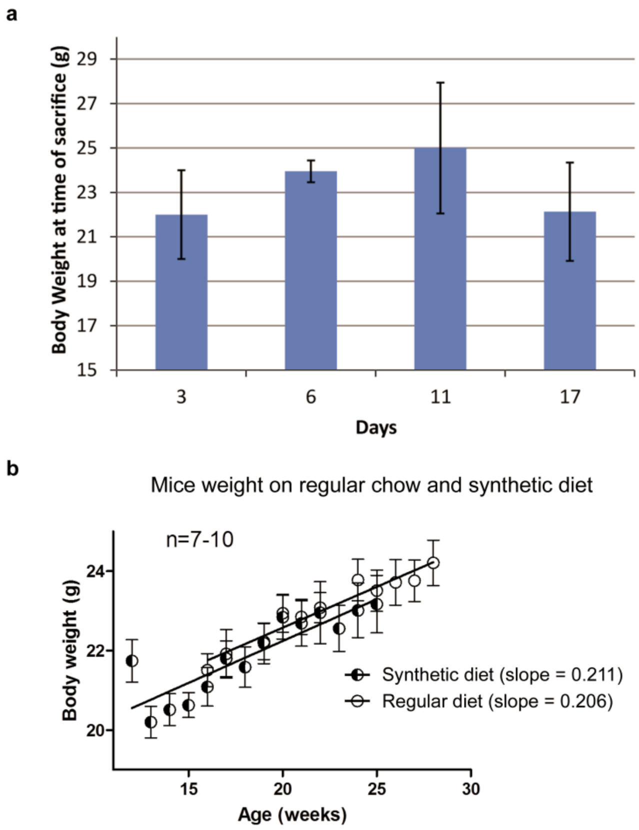


Supplementary Figure 2 – Validation of the amino acid precursor pool relative isotope abundance values calculated by Topograph. To test the accuracy of the precursor pool RIA values calculated by Topograph, yeast *Saccharomyces cerevisiae* cultures were grown to saturation in 50 ml of SC-Leu media supplemented with glucose and [5,5,5 – $^2H_3$] leucine at 33% or 67% enrichment levels. Cells were harvested, and the aqueous insoluble fraction digested and analyzed by LC-MS/MS. a) Histogram of amino acid RIA values from yeast cells grown in media enriched at 33%. The mean amino acid RIA calculated was 32.7% with a standard deviation of 8.3% and a median of 31%. b) Histogram of amino acid RIA values from yeast cells grown in media enriched at 67%. The mean amino acid RIA calculated was 67.5% with a standard deviation of 6.1% and a median of 67%.



Supplementary Figure 3 – Amino acid precursor pool RIA measurements are prone to greater error in spectra with low percentages of newly synthesized protein. An isotopologue distribution was generated, *in silico*, of a 3 tracer peptide with either 30% or 90% precursor pool RIA and newly synthesized peptide present at 10, 30, 60 or 90%. For each of these distributions, spectrum noise was simulated by randomly adding or subtracting 2% of the summed signal to each isotopologue. 1,000 simulated noisy spectra were generated and the precursor pool RIA was calculated by Topograph for each. a) Histograms of calculated precursor pool RIA values from simulated noisy isotopologue distributions at 30% precursor pool RIA. The median of all histograms were 30%. b) Histograms of calculated precursor pool RIA values from simulated noisy isotopologue distributions at 90% precursor pool RIA. The median of all histograms were

90%. The median of the precursor pool RIA values were all correct, however there is greater imprecision at lower percentages of newly synthesized protein as indicated by the widening distribution.



Supplementary Figure 4 – a) Body weight of mice at time of sacrifice. Mice were euthanized after 3, 6, 11 and 17 days on labeled synthetic diet (mean ± SD). b) Body weights of mice fed a regular diet (PicoLab Rodent Diet 20 #5053) or the synthetic diet. Mice were fed a regular diet until 11 weeks of age then switched to a synthetic diet from 12 to 25 weeks of age. An initial drop in body weight was observed as the mice adjusted to the new diet, but growth rates return to a rate comparable to mice kept on a regular diet.