

Figure 1. Dynamic proteomics workflow. (A) We have successfully used two isotopic labeling approaches to concurrently measure protein concentration and protein turnover. Turnover is measured by utilizing AA metabolism to isotopically label (purple) the tRNA-amino acid precursor pool (tRNA-AA). Protein concentration is measured through addition of exogenous labeled proteins (green). (B) Mice (or any other biosynthetic system, humans, etc) are labeled with $^2\text{H}_2\text{O}$ via bolus injection followed by intake in labeled drinking water. Samples are harvested, and protein homogenates, with addition of SILAM protein standards to selected samples, are separated by SDS-PAGE, followed by in-gel trypsinization. LC-MS/MS is then performed on tryptic peptides, and peptide isotopomer distributions are analyzed using mass isotopomer distribution analysis (MIDA) to quantify the fractional replacement (f) of hundreds or thousands of newly synthesized peptides and, thus, their parent proteins. The time dependence of f allows a fractional replacement rate (k) to be calculated. Using bioinformatics tools to highlight connections between proteins is highly informative and is used to identify and explain correlations in flux through the system. (C) For each peptide, ratios of SILAM-labeled peptides (green) to endogenous peptides (black) allow differences in protein concentrations to be measured. For clarity, a curve joining the peak of each isotopomer is shown. (D) Replacement of old, unlabeled proteins by newly synthesized proteins causes distinct changes in the isotope pattern of each peptide. These time dependent changes allow f and k to be measured for each protein from multiple peptides. For clarity, curves joining the peak of each mass isotopomer are shown.