Figure S1. Replicate analysis of the experiment to assess the level of non-specific binding proteins for enrichment of AHA-labeled proteins using alkyne resin-based click chemistry. Equal amounts of AHA-labeled or unlabeled (control) HEK cell lysates were used for the enrichment. Histograms of peptide intensities for the AHA labeled and the control samples are shown for the three replicates.
Figure S2. Replicate analysis of SILAC label incorporation for the BONLAC and pSILAC experiments. Two biological replicates were performed for each experiment.
Figure S3. Replicate analysis of correlation of protein intensities between the newly synthesized proteome (identified from the BONLAC experiment) and the steady state proteome (identified from the pSILAC experiment). Two biological replicates were performed for each experiment.
Figure S4. Replicate analysis of AHA-methionine replacement. Intensity ratio histograms for non-methionine containing peptides (blue) and methionine containing peptides (red) are shown. Normalization was performed so that the median ratio for non-methionine containing peptides is 1:1. Two biological replicates were performed for each experiment. In replicate 1, the median log2 ratio for methionine containing peptides is 0.637 (indicated by dashed line), which corresponds to an AHA:methionine ratio of 34:66 for BONLAC proteins. In replicate 2, the median log2 ratio for methionine containing peptides is 0.475 (indicated by dashed line), which corresponds to an AHA:methionine ratio of 28:72.
Figure S5. Replicate analysis to show BONCAT did not over- or under-represent methionine containing proteins. Two biological replicates were performed for each experiment. Distribution of identification frequency of proteins with different numbers of methionines is shown for the BONLAC and pSILAC experiments.