

**Combining selective fractionation techniques with SILAC
to enhance MS-based quantitative proteomics**

Ryan D. Bomgarden¹, Jennifer N. Sutton², Mary F. Lopez² and Paul Haney¹

¹Thermo Fisher Scientific, 3747 N. Meridian Rd., P.O. Box 117, Rockford, Illinois, USA 61105

²Thermo Fisher Scientific, 790 Memorial Dr., Suite 201, Cambridge, Massachusetts, USA 02139

(www.thermo.com/pierce)

Stable isotope labeling with amino acids in cell culture (SILAC) is a method for quantifying changes in the proteome by mass spectrometry (MS). Using this method to metabolically label A549 lung carcinoma cells, we successfully identified over 500 proteins from whole cell lysates and quantified proteins whose relative abundance changed in response to the anti-cancer compounds camptothecin and 17-AAG. However, only changes in the most abundant proteins were observed in whole cell lysates dictating the need for enrichment of lower abundant proteins with selective sample preparation techniques. Using a sulfhydryl-reactive biotinylation reagent, Biotin-HPDP, A549 cell lysates were enriched for cysteine-containing proteins before MS analysis. This enrichment step resulted in the identification and quantitation of additional proteins whose levels were altered after drug treatment. In an alternative approach, proteins of heavy isotope-labeled A459 cells treated with the nuclear export inhibitor leptomycin B (LMB) were enriched in nuclear extracts before MS analysis in order to identify known and novel exportin/CRM1-dependent proteins. MS spectra of isotopic peptide pairs were compared and used in SEQUEST database searches to identify proteins whose relative abundance was changed after treatment. This analysis resulted in the identification and quantitation of over 600 proteins, which were highly enriched for nuclear proteins. Both of these model systems show the utility of combining selective fractionation techniques with MS-based analysis to identify and quantify proteins using stable isotope incorporation through metabolic labeling.