Chemotaxis and toll-like receptor (TLR) signaling in macrophages are critical to the immune response. To model these signaling networks, we are using selected reaction monitoring (SRM) to measure the absolute abundance of pathway proteins, and the resulting values are being used as pathway model parameters. RNA-seq was performed to identify expressed transcripts, and shotgun mass spectrometry was used to identify proteotypic peptides. SRM using heavy-labeled internal peptide standards was used to quantify the chemotaxis pathway proteins. The transcript and protein abundance values correlated strongly, and estimated protein abundance values for the entire macrophage proteome were produced. Computational modeling of the chemotaxis pathway was performed using Simmune modeling tool. Molecular reaction rates were not directly measured, but instead were constrained using a model training dataset consisting of multiple types of in vitro microscopy data. Subsequently, model testing used an orthogonal dataset from ELISA assays of RAC1-GTP and RHOA-GTP.

The model produced in silico results consistent with both the training and testing datasets, and it was determined to be robust by assessing the accuracy of thousands of perturbed models. These findings demonstrate the feasibility and value of combining mass spectrometry-based measurements with pathway modeling for advancing biological insight. SRM assays for the canonical TLR signaling pathway and related proteins have been successfully developed. In parallel, a preliminary model of the TLR pathway has been developed using the estimated protein abundance values. The relative proteome and secretome measurements for cells stimulated with TLR ligands and the cytokine secretion values measured by flow cytometry and ELISA are being used as additional constraints for this complex model. Our findings demonstrate the feasibility and value of combining mass spectrometry-based measurements with pathway modeling for advancing biological insight.

**BIO**

Dr. Nita-Lazar received her Ph.D. in biochemistry in 2003 from the University of Basel for studies performed at the Friedrich Miescher Institute for Biomedical Research, where she analyzed protein glycosylation using mass spectrometry methods. After postdoctoral training at Stony Brook University and Massachusetts Institute of Technology, where she continued to investigate post-translational protein modifications and their influence on cell signaling, she joined the Program in Systems Immunology and Infectious Disease Research, now the Laboratory of Systems Biology, in April 2009.