Title: Quantitative Measurement of Full-Length and C-Terminal Proteolyzed RBP4 from Serum using mass spectrometric immunoassay with high resolution and accurate mass detection (MSIA-HRAM)

Bryan Krastins1, Gregory Byram1, David Sarracino1, Wendy Zhang2, Andre Kopoyan2, Sunny Tam2, Mary Lopez1

1 – Thermo Fisher Scientific, BRIMS Center, 790 Memorial Drive, Cambridge, MA 02139

2- Nuclea Biotechnologies, 46 Elm Street, Pittsfield, MA 01201

Abstract:

Serum retinol-binding protein 4 (RBP4) levels have been associated with diabetes and insulin resistance. In other studies, it was found to be associated with Congenital Heart Defect (CHD). It is potentially one of the Risk Factors for Ischemic Stroke in Women. Two major proteolyzed forms of RBP4 in human serum (RBP4-L and RBP4-LL) account for more than 50% of total RBP4 levels in some insulin resistant individuals. RBP4-L correlates highly with insulin resistance, but RBP4-LL does not. The presence and level of expression of these 3 forms of RBP4 might offer a highly specific and sensitive biomarker assay for diabetes, heart diseases and other conditions.

Currently, western analysis and ELISA do not provide high throughput or specific assay to quantify these forms of the same protein for clinical samples. Hence, we have applied a novel mass spectrometric immunoassay with high resolution and accurate mass detection (MSIA-HRAM) to address this short coming from human plasma samples. This approach allows proteins to be tested in biological samples in a high-throughput and semi-automated manner. It is also applicable to the discovery and measurement of protein modifications that may be biologically significant. In MSIA, antibody-derived affinity tips are utilized to capture and enrich the RBP4 protein. After the captured RBP4 protein is eluted from the tips, the protein is digested with Lys-C and analyzed by a Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer. Heavy isotope-labeled versions of target peptides were used as internal standards. Pinpoint software was used for targeted protein quantification.