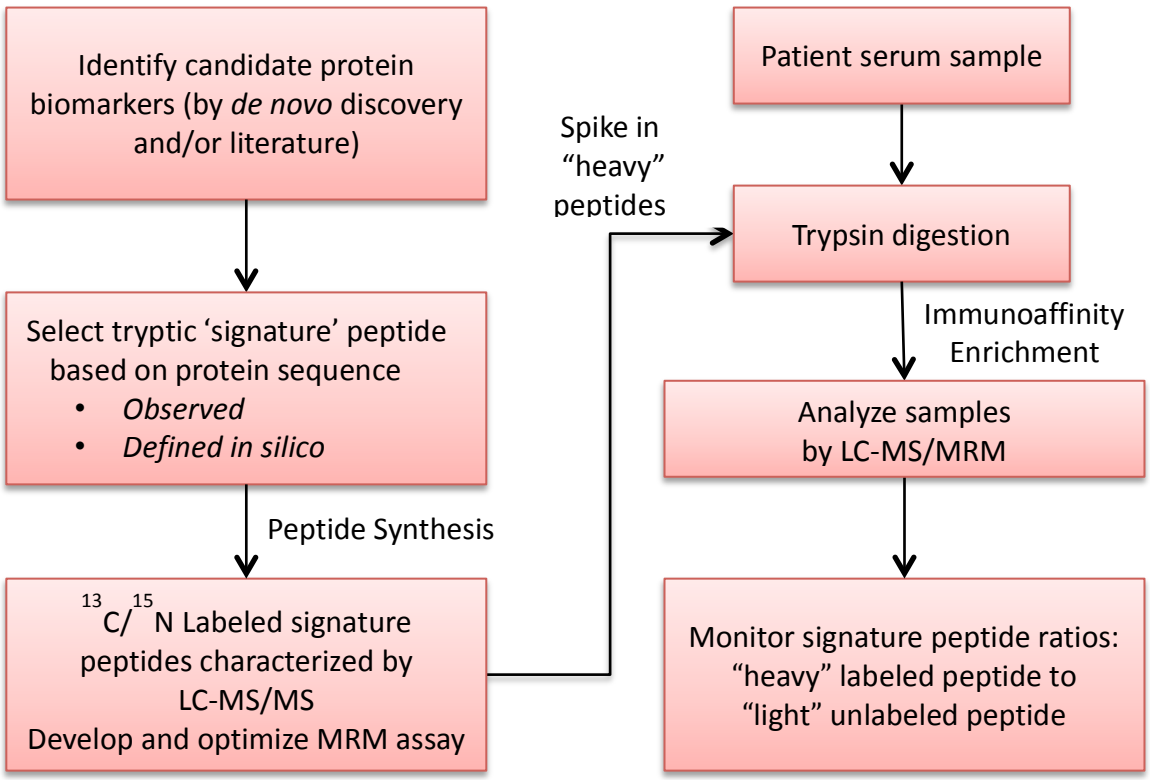
SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Antibodies)

SISCAPA[1] technology is one of a number of emerging immuneaffininty mass spectrometry approaches for quantitation of protein biomarkers in serum/plasma.[2-3] SISCAPA assays combine the precision of MRM mass spectrometry with the power of affinity enrichment to deliver an alternative to conventional immunoassays for protein quantitation. The method exploits anti-peptide antibodies for enrichment of target proteotypic peptides in a complex sample wherein the captured peptides are quantified by MRM relative to stable isotope labeled synthetic peptides as internal standards using a triple quadrupole (QQQ) mass spectrometer. Capitalizing upon the selectivity of anti-peptide antibodies, the SISCAPA approach affords an improvement in sensitivity of from 100- to 1000-fold with a single enrichment step, and without the need for depletion of abundant proteins or extensive sample fractionation.

The workflow consists of identifying one or more "signature peptides" unique to the candidate biomarker to be validated either *in silico* or observed during the discovery phase of the biomarker development pipeline. The corresponding stable-isotope labeled synthetic standards (SIS) are synthesized and characterized by LC/MS/MS. The transitions associated with both the "heavy" stable-isotope labeled and endogenous "light" unlabeled peptide are optimized for the MRM assay. Preferably plasma or serum samples are subjected to standard tryptic digestion followed by spiking of known concentrations of the SIS. Anti-peptide antibodies against the target peptides are immobilized on magnetic beads that are added to the digested samples and allowed to capture the target peptides. This is followed by washing to remove unretained peptides, elution of captured peptides from the magnetic beads, and analysis by LC-MRM-MS. In the proteomics core facility, this workflow uses the Agilent 1260 Infinity HPLC-Chip Cube MS interface in conjunction with the Agilent 6490 iFunnel triple quadrupole LC/MS system. The concentration of the endogenous target peptide enriched from the sample digest is determined by comparing the peak area ratios of the unlabeled peptide to that of the SIS.



The SISCAPA approach has great utility for biomarker verification. Although ELISA remains the current gold standard for protein biomarker measurement, the development of *de novo* immunoassays for a large number of biomarkers remains impractical due to lack of paired specific antibodies, long lead times, and the high cost associated with the generation of high quality monoclonal antibodies. Furthermore, ELISA measures the target protein primarily in its free form and does not account for the target protein bound to autoantibodies or other tightly bound protein complexes. Alternatively, the anti-peptide antibodies used in the SISCAPA approach recognize linear peptide epitopes and are insensitive to intact protein tertiary structure, activity, and complex formation. Furthermore, in many instances polyclonal antibodies have exhibited utility for SISCAPA. Because the antibodies bind both the labeled and unlabeled target peptides equally, the quantitative information is preserved throughout the enrichment process and reflects the total protein concentration in the sample. The SISCAPA approach achieves limits of quantification (LOQ) of proteins in the low ng/mL range from 10 µL of plasma and low pg/mL range from 1mL of plasma. This immunoaffinity-based approach can be used to test a large number of candidate biomarkers in a large number of patient samples by automating the method thereby improving the throughput and performance.

1. Anderson, N.L. et al. (2004) Mass Spectrometric Quantitation of Peptides and Proteins Using Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA). *J. Proteome Research* 3, 235-244
2. Razavi, M. et al. (2012) High-throughput SISCAPA quantitation of peptides from human plasma digests by ultrafast, liquid chromatography-free mass spectrometry. *J Proteome Research* 11, 5642–5649
3. Krastins, B. et al. (2013) Rapid development of sensitive, high-throughput, quantitative and highly selective mass spectrometric targeted immunoassays for clinically important proteins in human plasma and serum. *Clin. Biochem.* 46, 399-410