Principal Investigator: Sergey Vakhrushev
Grant Title: Developing the Next-Generation of O-Glycoproteomics
Abstract:

In O-glycoproteomics the analysis of O-glycan structures is well established while localization of O-glycans on proteins offers considerable challenges. We have recently made a significant breakthrough in the discovery of localization of O-glycosites by developing a strategy based on mass spectrometry applied to genetically engineered cells, so-called “SimpleCells” (SC). In SC virtually all GalNAc-type (mucin-type) O-glycans in these cells are truncated to GalNAc (Tn) or NeuAc-GalNAc (SiaTn), which then carry the site information through a straightforward shotgun high resolution mass spectrometry facilitated by ETD-MS2 fragmentation preserving intact the glycan-peptide linkage. The major obstacle now is to overcome the difficulty of simultaneous identification of native O-glycan structures and glycosites. **The objectives** of the proposal were: 1) Develop a strategy that will enable high throughput O-glycoproteomics screening of complex samples with simultaneous identification native O-glycostructures and O-glycosites 2) Apply this strategy for O-glycoproteomics screening of "wild type" (WT) samples. **The methods** used for the first aim were lectin affinity chromatography (LWAC) to enrich for O-glycopeptides, Delphi and Visual Basic for script programming and Microsoft Visual Studio to develop O-glycoproteomics data base (DB). For the second aim we used LC-MS OrbiTrap equipped with ETD for glycoproteomics screening and Proteome Discoverer 1.4 Software for data processing. **The results** of these experiment showed that sequential use of a panel of lectins could simultaneously enrich O-glycopeptides bearing T-, Tn, and O-Man epitopes. We have developed a script predicting O-glycopeptides from WT sample using SC O-glycoproteomics DB alignment. All these provided an opportunity to perform O-glycoproteomics of bio-fluids and tissue samples. We have probed our strategy with human urine and mouse brain and achieved ultra deep level of identification per single sample. Approximately 1000 O-glycoproteins for human urine and around 500 O-glycoproteins for the mouse brain. We have increased human O-glycoproteome SC DB capacity by repeating 12x human SC lines with another enzyme, such as a chymotrypsin and reprocessing previously published and new data with semi-specific enzymatic digestion. As a result of these experiment the total number of O-glycoproteins in a DB raised from previously published 600 to a nearly 3000.