Descriptions of New Technologies Available in the

Proteomics Core of the NIDA Neuroproteomics Center

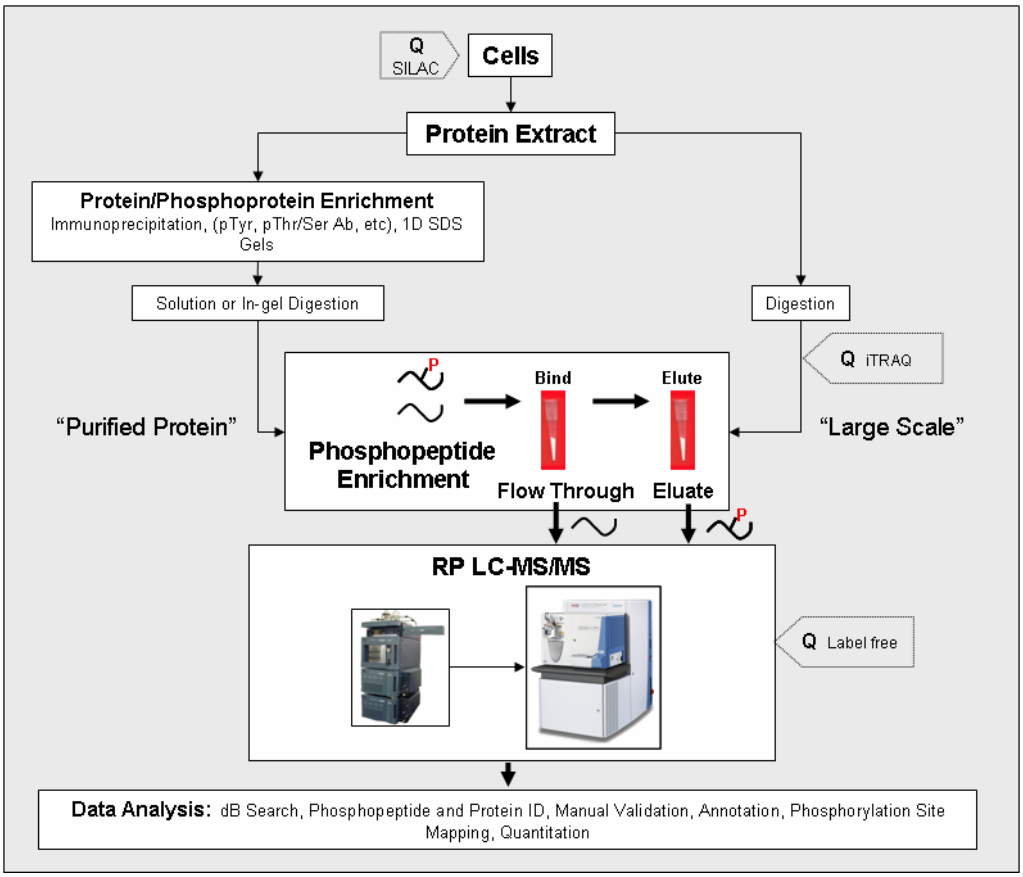
nano-UPLC-LTQ Orbitrap Mass Spectrometer Platform for Phosphoproteomics

Description

Combining our in-house developed phosphopeptide enrichment methodologies, a state-of-the-art nanoACQUITY™ Ultra Performance or Ultra-high pressure, LC (UPLC) system manufactured by Waters Corp. coupled with the state-of-the-art LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific), we now offer a platform to provide what we believe to be the first robust, dedicated phosphoprotein profiling and related phosphoproteomics services available from an academic core facility. The Figure below schematically highlights few of the various workflows that are briefly discussed.

Phosphoproteomics is an area where the Keck Lab has made substantial progress over the last year with regard to optimizing the tryptic phosphopeptide enrichment methodologies that are carried out prior to quantitative proteomic analysis and that are so critically important for detecting the very low level of in vivo phosphorylation that is so often found at any given peptide site. It has been estimated that one third of mammalian proteins may be phosphorylated (Hubbard and Cohen, 1993) and this modification often plays a key role in modulating protein function. Since so little data is available regarding differential extents of protein phosphorylation in response to human disease, we believe this is a very promising area of fundamental, translational and even clinical research and relates directly to the use of phosphoproteins as emerging biomarkers (Moran et al., 2006, and Lim, 2005).

The nanoACQUITY UPLC system supplies non-split gradient nanoflow for high resolution chromatographic separations optimized for small 75µm diameter columns that have uniform non-porous silica based spherical beads 2 µm and less in diameter. This configuration not only significantly reduces the time it takes to run a single chromatographic separation (De Villiers et al., 2006; Jones and Plumb, 2006), but it also increases detection limits of electrospray ionized samples from the higher concentrations of eluents under much narrower eluting liquid chromatographic peaks. This instrument is coupled on line in front of our newly acquired LTQ-Orbitrap mass spectrometer which has a unique, and also very fast MS^2 and MS^3 data dependent phosphopeptide acquisition capability. The complementary fast analysis speed of the integrated system tightly coupled with the robust, highly sensitive, fast scanning, high resolution, high mass accuracy, and wide dynamic range characteristics of the LTQ-Orbitrap together create an unmatched ability to confidently detect and identify large numbers of phosphopeptides from highly complex, digested cell and tissue protein extracts of interest.



Steps to MS based Phosphoproteomics:

1. <u>Phosphopeptide</u>

Enrichment: The first step requirement to most MS based phosphoproteomic analysis is the affinity enrichment of phosphopeptides. Currently our core uses several approaches. One approach is based on the use of strong cation exchange chromatography (SCX) to enrich and fractionate for early eluting phosphopeptides and another very robust approach uses TiO_2 as the affinity support. Both of these approaches can be combined for more effective enrichment. The specific method of choice will be discusses with the individual investigators for optimum experimental outcome.

2. Identification of sites of phosphorylation: Allows LC-MS/MS analysis of enriched phosphopeptides from affinity purified phosphoproteins for the discovery or verification of sites of phosphorylation.
   a. ***Sample Requirements:*** *50ng or more proteins of interest purified on a gel band or immunoprecipitated in solution with minimal interference of antibody proteins*
3. Large Scale phosphoprotein Profiling: Uses both SCX fractionation and TiO_2 affinity based phosphopeptide enrichment methodologies to identify large number of phosphopeptides from complex protein extracts using our newly acquired Thermo Fisher LTQ-Orbitrap mass spectrometer.
   a. ***Sample Requirements:*** *For identification of optimum number of phosphopeptides, 1 mg or more starting protein amounts are preferred. However, as low as 200 μg of starting protein material can be accepted.*
4. Relative quantitation of phosphorylation: Using already established quantitative protein profiling techniques like SILAC, iTRAQ and Label free Quantitation, relative abundance of phosphorylation between different samples can be compared. The choice of these techniques are strongly dependent on the nature of the experimental systems being used
   a. ***Sample Requirements:*** *Investigators starting on a quantitative experimental design for determining changes on level of phosphorylation are strongly encouraged to consult with Keck Staff before deciding on any experimental approaches. Similar guidelines can be followed from above on the amount of samples required to conduct quantitation experiments.*

Contact Person: Erol E. Gulcicek, Ph.D.
Co-Director, Protein Profiling
(203) 785-6296; erol.gulcicek@yale.edu