

High Throughput Proteomics Analyses Enabled by Q Exactive Technology

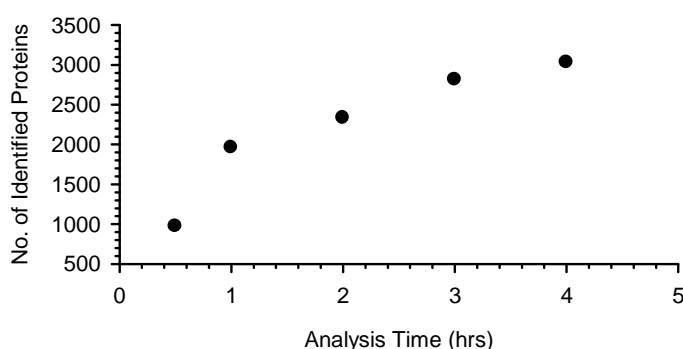
The Thermo Scientific Q Exactive is a hybrid quadrupole-Orbitrap mass spectrometer combining a high-performance quadrupole mass filter with the industry leading high resolution sensitive and selective Orbitrap mass analyzer. The data below were generated from the analysis of 1µg of a K562 cell lysate. Each sample was analyzed in triplicate. The K562 tryptic digest was analyzed using 0.5, 1, 2, 3 and 4hr reverse phase gradients.

Experimental Details

The tryptic digest was analyzed by nano LC/MS/MS with a Waters NanoAcquity HPLC system interfaced to a ThermoFisher Q Exactive mass spectrometer. Peptides were loaded on a trapping column and eluted over a 75µm analytical column at 350nL/min; both columns were packed with Jupiter Proteo resin (Phenomenex). Gradients of 0.5, 1, 2, 3 and 4h were employed in analytical triplicate. The mass spectrometer was operated in data-dependent mode, with MS performed at 70,000 FWHM resolution and MS/MS performed at 17,500 FWHM resolution. The fifteen most abundant ions were selected for MS/MS. Data were searched using a local copy of MaxQuant 1.2.2.5 with the Andromeda search engine.

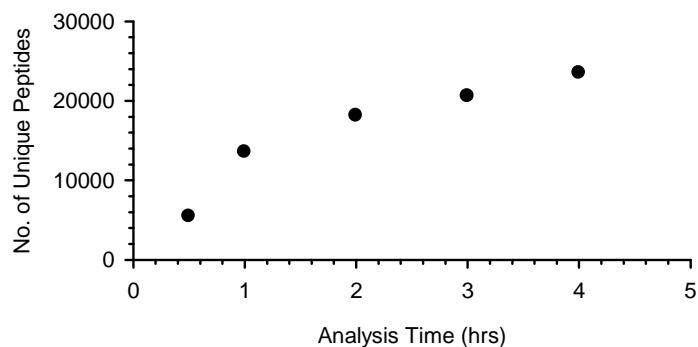
Results

The charts below show the number of proteins, unique peptides and PSMs identified as a function of analysis time.

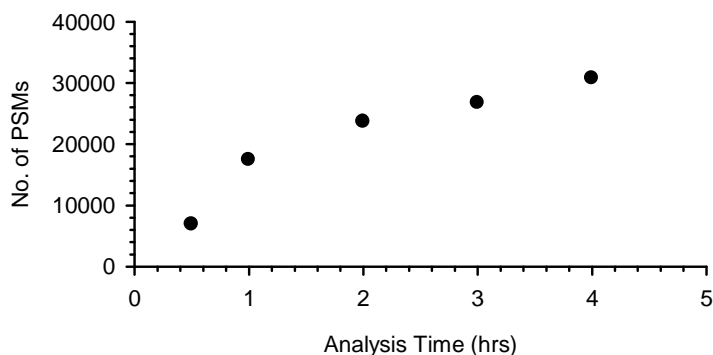


Time (hrs)	0.5		1		2		3		4	
	average	% CV	average	% CV	average	% CV	average	% CV	average	% CV
# identified Proteins*	970.5	3.0	1960	0	2332	1.3	2812.5	2.1	3028.5	2.2

*with two unique peptides per protein



Time (hrs)	0.5		1		2		3		4	
	average	% CV	average	% CV	average	% CV	average	% CV	average	% CV
Unique Peptides	5458	2.9	13529	0.4	18101.5	0.5	20563	3.4	23496	0.3



Time (hrs)	0.5		1		2		3		4	
	average	% CV	average	% CV	average	% CV	average	% CV	average	% CV
PSMs**	6865.5	5.7	17392	0.8	23648	0.5	26684	6.1	30725.5	0.3

** A PSM is any spectrum matching to a peptide and includes repeat observations and PTM-modified peptides