

## 6x Nanoflow ultra-high-pressure liquid chromatography (UPLC)

### Functional Requirements

These flexible, state-of-the-art, high end nanoflow chromatography systems will be used to analyse primarily, albeit not exclusively, proteolytic peptides from complex biological samples by C18 liquid chromatography before mass spectrometry (MS), with the possibility to (but not necessarily limited to):

- Shotgun proteomics experiments of proteolytic peptides (not limited to trypsin) on high-end mass spectrometers. The nanoLC-MS setup will focus on the analysis of proteolytic peptides, phosphopeptides, glycopeptides and peptides bearing other PTMs. In addition, large peptides originating from alternative enzymes or short incubations will be separated.
- Analysis of peptides from limited sample material and/or containing PTMs requires high sensitivity. Therefore, the instrument must be very stable for an extended period of time at nanoflow rates of ~100nL/min with high retention time precision.
- The nanoLC system will be coupled to existing MS instrumentation present in the research group and both the nanoLC and MS should be operatable from a single software program, allowing seamless scheduling and timing of nanoLC gradient and MS instrument settings.
- MS acquisition software used in the research group be able to directly read out LC performance during LC-MS operation.
- Must include 4-year preventive maintenance (PM) contract, including PM consumables, after the guarantee period.

### Technical Requirements

The scientific requirements above can be translated to minimal instrument requirements for the chromatographic separation system for proteolytic peptides and modified peptides (e.g. phosphopeptides):

#### NanoLC system

- precise flow control and high retention time precision; RSD  $\leq$  0.2%
- Flow range: 50-1500 nL/min
- Maximum LC pressure:  $\geq$  800 bar at full flow range
- Direct continuous flow in preconcentration setup
- Integrated loading pump with a flow range of 5  $\mu$ L up to 2.5mL / minute

- Fast sample loading onto the trap column (~5-10 seconds)
- Quick fingertight, zero-dead volume and leak-free connections between LC modules, valves, and columns.
- Integrated solution for nanoLC column, column-heater and nanospray source
- Nanospray source must be able to operate with stable spray for extended duration
- Nanospray source must be compatible with flow rates of 50 nL/min to 1  $\mu$ L/min