CURRENT NANO-LC-MS/MS PROTOCOL: NCBS – MS FACILITY

Instruments:

1200, 1D nano-LC (Agilent)

Nanomate Triversa (Advion)

LTQ – Orbitrap Discovery (Thermo)

Sonicator

Chemicals:

Water (LC/MS grade-Fluka)

Acetonitrile (LC/MS grade –Fluka)

Formic Acid (LC/MS grade - Fluka)

Consumables:

Glass tips (Sigma)

Protein low bind tubes and tips (Eppendorf)

Autosampler vials (Agilent)

Brief Introduction of instruments and standard operating protocol and checkpoints:

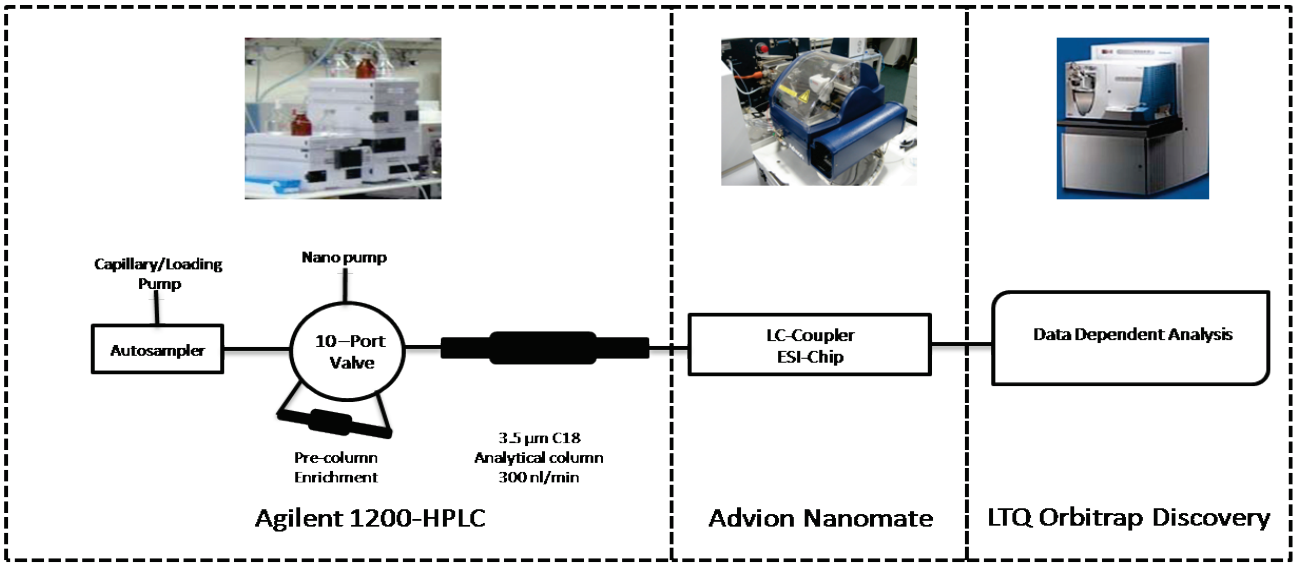

Fig 1) LCMS/MS set up at MS Lab

1) Agilent 1200 HPLC:

High performance liquid chromatography enables to separate complex tryptic peptide mixture. We are using a 1D nano-LC setup consisting of a capillary pump for loading the sample (several microliter volume, operated at 20µL/min) onto a trap column and a nano pump (operated at 300nL/min) for the separation on the analytical column (see Fig 1). We use 100% water with 0.1% formic acid as solvent A and 80% Acetonitrile + 20% water with 0.1% formic acid as solvent B. We utilize three standard gradients of 70, 110 and 180 minutes length depending on the complexity of the sample (2, see below). The usual pressure profile of the LC-system is shown in Fig 2.

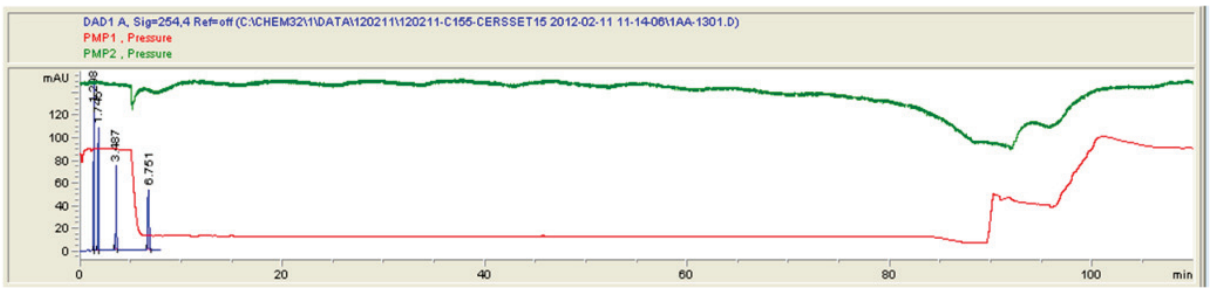

Fig 2) Pressure profile of capillary and nano pump for 110 minute standard gradient.

Solvent preparation:

For formic acids use only washed glass pipettes/ glass capillaries. Wash them with LC/MS grade water 3 times followed by wash with 50% Acetonitrile 3 times and last with 100% Acetonitrile 3 times. Let it dry for some time then use it for pipetting acids.

Solvent A: 100 % water with 0.1% Formic Acid:

Take new 1liter bottle of LC/MS grade water and add 1.0ml of Formic acid into that sonicate and degas for 15 min. Then replace old bottle with new one. The remaining solvent from the old bottle might be added into capillary pump solvent A bottle after inspection.

Solvent B: 80%acetonitrile + 20% water with 0.1%formic acid:

Measure the solvents in the specified glass cylinder which is washed previously as mentioned above for pipette and make 80% Acetonitrile in water with 0.1%formic acid.

Sample preparation for mass spectrometry:

After tryptic digestion reconstitute the peptides in 0.1% formic acid vortex for 1hr followed by centrifugation at 10,000 rpm for 5 min. Collect supernatant into Agilent auto sampler vials and inject required volume. Care should be taken to avoid particles in the sample vials it may clog the flow path.

Checkpoints for Operating Agilent 1200 – HPLC:

1. At the start of a standard gradient the pressure of the nano pump should be at 140-150bars and the capillary pump be 85-95bars. The pressure profile of a run should be similar to Fig 2.
2. The auto sampler temperature should be 4°C.
3. Control reservoir for wash pump for the autosampler. The injector needle with 50% acetonitril in water (both solvents of HPLC grade)
4. At the end of sequence the pressure profile should look like as shown in Fig (2).
5. Update the Antivirus software once in 2-3 weeks.

Standard gradients 1D nano-LC MS/MS at NCBS MS-Facility:

Table 1) Gradients for nano-pump

Nano pump				
S.No	%B	Time (180 min)	Time (110 min)	Time (70 min)
1	11	0	0	0
2	11	5	5	5
3	25	79	45	25
4	53	138	77	41
5	100	156	86	46
6	100	160	90	50
7	11	164	94	54
8	11	180	110	70

Table 2) Gradients for capillary pump

Capillary pump				
S.No	%B	Time (180 min)	Time (110 min)	Time (70 min)
1	1	0	0	0
2	1	5.5	5.5	5.5
3	50	8	8	8
4	50	154	80	49
5	100	159	89	50
6	100	163	93	53
7	1	165	95	55
8	1	180	110	70

10-port valve switching from position 1 to position 2 at 5th minute.
In 180 min gradient valve switching from psotion 2 to postion 1 at 160th minute.
In 110 min gradient valve switching from psotion 2 to postion 1 at 90th minute.
In 70 min gradient valve switching from psotion 2 to postion 1 at 50th minute.

2) Advion Nanomate Triversa:

The Nanomate Triversa is used as an automated nano-ESI source to generate ions for later mass spectrometric analysis. A specifically designed LC coupler connects the flow from the LC to the ESI chip where the nano-ESI generated ions are transferred into the mass spectrometer. The LC-coupler has a limited lifetime of approximately 300 hundred runs. The spray current is monitored and should at the beginning of a standard gradient be above 50nA. With help of the spray sensing mode the LC-MS/MS acquisitions of a sequence can be monitored.

Checkpoints for Operating Advion Nanomate:

1. After starting the instrument check for the current profile. The current should not be below 50 nA at the start of a standard LC-gradient.
2. Control position of nozzle and LC-coupler to the ion transfer capillary.

3) LTQ – Orbitrap Discovery:

LTQ Orbitrap Discovery is a hybrid type MS system with the ability to determine accurate m/z of intact precursors in the orbitrap analyzer. The two dimensional iontrap achieves high scan rates ideal suited for fast MS^n analysis in LC time domain.

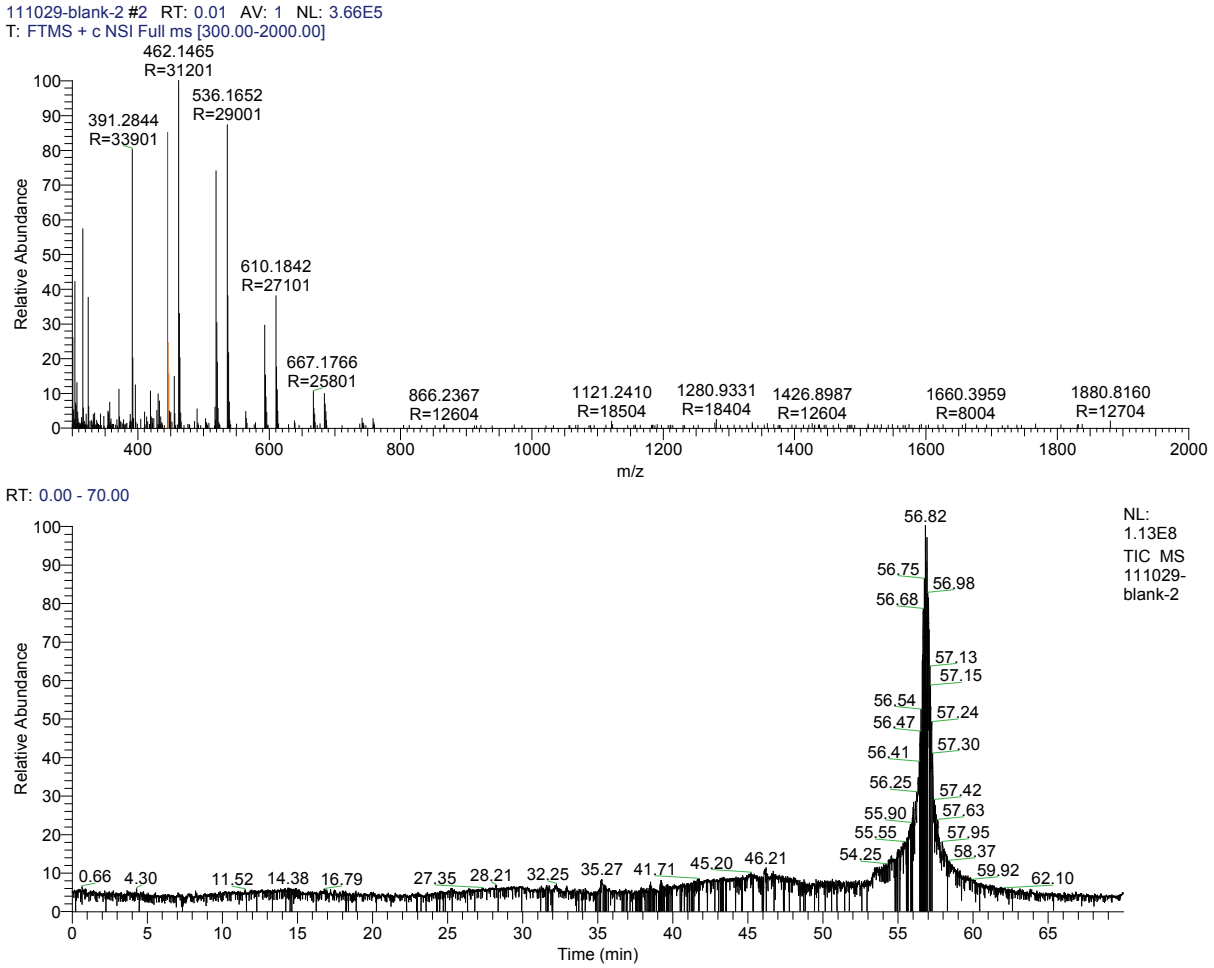

Fig.4) Representative spectrum as usually detected at the beginning of a standard gradient. The major signals are corresponding to polysiloxanes. Typical Total Ion Count (TIC) of a blank injection.

Standard Instrument methods:

Run time: 70, 110 an 180 min.
MS scan/survey scan is taken in FT mode at 30,000 FWHM and MS/MS in the linear ion trap.
MS1 mass range 300-2000 with lock mass: 445.1200 (corresponding to polysiloxane),
DDA settings: 1 MS survey scan followed by 5 MS/MS scans, exclusion duration120.0sec
Conditions to trigger MS/MS: minimum signal intensity <10,000; charge state is: +2, +3,
Activation type: CID,
Maximum injection time for MS/MS: 500ms
Isolation width: 2 amu,
Normalized collision Energy: 35

Checkpoints for Operating of LTQ-Orbitrap Discovery:

1. Check for the lock mass error which ideally should be less than 10 ppm if not calibrate the instrument.
2. Check the TIC and spectra of blank sample as shown in fig 4.
3. Update the Antivirus once in 2-3 weeks.

Reference:

1. Olsen, J. V.; de Godoy, L. M. F.; Li, G. Q.; Macek, B.; Mortensen, P.; Pesch, R.; Makarov, A.; Lange, O.; Horning, S.; Mann, M. Mol. Cell. Proteomics 2005, 4, 2010– 2021.

2. Junqueira M, Spirin V, Balbuena TS, Thomas H, Adzhubei I, Sunyaev S, Shevchenko A. Protein identification pipeline for the homology-driven proteomics. J Proteomics. 2008 Aug 21;71(3):346-56.