

Mass spectrometry

1. PROTEIN DIGESTION

100 µg of protein per condition was transferred into a new Eppendorf tube and the final volume was adjusted to 100 µL with 8 M urea. 2 µL of 0.5 M TCEP was added and the sample was incubated at 37°C for 1 h, and then 4 µL of 1 M iodoacetamide was added to the sample and the incubation was last for 40 minutes protected from light at room temperature. After that, five volumes of -20°C pre-chilled acetone was added to precipitate the proteins overnight at -20°C. The precipitates were washed by 1 mL pre-chilled 90% acetone aqueous solution for twice and then re-dissolved in 100 µL 100 mM TEAB. Sequence grade modified trypsin (Promega, Madison, WI) was added at the ratio of 1:50 (enzyme : protein, weight : weight) to digest the proteins at 37°C overnight. The peptide mixture was desalted by C18 ZipTip and then lyophilized by SpeedVac.

2. nano-HPLC-MS/MS ANALYSIS

The sample was analyzed by on-line nanospray LC-MS/MS on Orbitrap Fusion™ mass spectrometer (Thermo Fisher Scientific, MA, USA) coupled to an EASY-nanoLC 1000 system(Thermo Fisher Scientific, MA, USA) . 1 µL peptide was loaded (analytical column: Acclaim PepMap C18, 75 µm x 25 cm) and separated with a 60 min gradient. The column flow rate was maintained at 600 nL/min with the column temperature of 40°C. The electrospray voltage of 2 kV versus the inlet of the mass spectrometer was used. The chromatographic gradient is shown below (A: 0.1% formic acid in water; 0.1% formic acid in CAN):

Time	Duration	Flow [nl/min]	Mixture [%B]
00:00	00:00	600	5
03:00	03:00	600	8
37:00	34:00	600	20
51:00	14:00	600	30
52:00	01:00	600	90
60:00	08:00	600	90

The mass spectrometer was run under data dependent acquisition mode, and automatically switched between MS and MS/MS mode. The parameters was: (1) MS: scan range (m/z)=350–1550; resolution=60,000; AGC target=4e5; maximum injection time=50 ms; include charge states=2-6 (2) HCD-MS/MS: resolution=15,000; isolation window=1.6; AGC target=5e4; maximum injection time=120 ms; collision energy=35.