

Supplementary Data 12: MS Settings.

Project	MS	general	MS1	MS2	MS2	MS3	Comments; special settings
ACE_0353 ACE_0371	Elite	TunePlus v2.7.0 SP1 Gradient: 70 min	Analyzer: FT Res.: 60000 SR: 350 - 1500 AGC: 1×10 ⁶ AcT: 50 DDM: NS	Analyzer: FT Res.: 15000 SR: variable AGC: 1×10 ⁵ AcT: 120 ms CS: >+2 Frag.: CID NCE: 35 NS: 5×	Analyzer: FT Res.: 15000 SR: variable AGC: 1×10 ⁵ AcT: 120 ms CS: >+2 Frag.: ETD NCE: n/a NS: 5×		5 th order double play. MS2 was done in OT. Each mass selected for fragmentation was first fragmented by HCD and then by ETD. MS2's were only performed on charge states >+2.
ACE_0372-01	Elite	TunePlus v2.7.0 SP1 Gradient: 140 min	Analyzer: FT Res.: 120000 SR: 300 - 2000 AGC: 1×10 ⁶ AcT: 70 DDM: NS	Analyzer: FT Res.: 15000 SR: variable AGC: 5×10 ⁵ AcT: 250 ms CS: >+2 Frag.: HCD NCE: 31 NS: 10×			MS2 was done in OT; MS2 was performed only on charge states >+2.
ACE_0372-02	Elite	TunePlus v2.7.0 SP1 Gradient: 140 min	Analyzer: FT Res.: 60000 SR: 350 - 2000 AGC: 1×10 ⁶ AcT: 70 DDM: NS	Analyzer: FT Res.: 15000 SR: variable AGC: 5×10 ⁵ AcT: 250 ms CS: >+2 Frag.: CID NCE: 35 NS: 5×	Analyzer: FT Res.: 15000 SR: variable AGC: 5×10 ⁵ AcT: 250 ms CS: >+2 Frag.: ETD NCE: n/a NS: 5×		5 th order double play. MS2 was done in OT. Each mass selected for fragmentation was first fragmented by HCD and then by ETD. MS2's were only performed on charge states >+2.
ACE_0393	Elite	TunePlus v2.7.0 SP1 Gradient: 70 min	Analyzer: FT Res.: 120000 SR: 300 - 2000 AGC: 1×10 ⁶ AcT: 70 DDM: NS	Analyzer: FT Res.: 15000 SR: variable AGC: 5×10 ⁵ AcT: 250 ms CS: >+2 Frag.: HCD NCE: 31 NS: 10×			MS2 was done in OT; MS2 was performed only on charge states >+2.

ACE_0422 MS2-MS2- MS3	Lumos	Tune v3.1.2412.25 Gradient: 60 min	Analyzer: FT Res.: 120000 SR: 375 - 1600 AGC: standard AcT: 50 RF: 30 SF: 10V DDM: CT/5sec	Analyzer: FT Res.: 30000 SR: Auto AGC: Auto AcT: 100 ms CS: >+2 IsM: Q IsW: 1.6 (MS) Frag.: CID NCE: 25	Analyzer: FT Res.: 50000 SR: Auto AGC: 400% AcT: 150 ms CS: >+2 IsM: Q IsW: 1.6 (MS) Frag.: EThcD NCE: 20 NS: 1	Analyzer: IT Res.: 15000 SR: Auto AGC: 200% AcT: 120 ms CS: >+2 IsM: IT IsW: 2.5 (MS) 2 (MS2) Frag.: HCD NCE: 35 NS: 4	First MS2 is triggered by targeted mass difference 31.9721 (DSSO). Second MS2 is performed in OT with EThcD. Fragments from first MS2 with triggered mass difference were collected for MS3 experiments (HCD in IT). The whole sequence MS1-MS2-MS2/MS3 was repeated for 5 sec (cycle time between master scans).
ACE_0422 ACE_0425 MS2- stepped HCD	Lumos	Tune v3.1.2412.25 Gradient: 60 min	Analyzer: FT Res.: 120000 SR: 375 - 1600 AGC: Standard AcT: 50 RF: 30 SF: 10V DDM: CT/5sec	Analyzer: FT Res.: 30000 SR: Auto AGC: Standard AcT: 150 ms CS: >+2 IsM: Q IsW: 1.6 (MS) Frag.: sHCD NCE: 21,27,33			MS2 is performed on all ions with charge >+2. Fragmentation type is stepped HCD (sHCD). Each 1/3 of AGC will be separately treated with predefined increasing NCE (here 21, 27, 33). The ions are collected in the IRM. Once all portions were fragmented the whole batch is send to the OT for analysis at the same time. Advantage of this approach ... at low NCE the cross linker (DSSO) cleaves ... and releases the signature peptides .. at higher fragmentation these arms will be fragmented. So we have diagnostic peaks in the spectrum (which can be nicely used to identify CL spectra) and fragments from the arms for identification.
ACE_0556	Lumos	Tune v v3.3.2782.28 Gradient: 160 min	Analyzer: FT Res.: 120000 SR: 400 - 1600 AGC: 150% AcT: 60 RF: 30 SF: -- DDM: CT/5sec	Analyzer: FT Res.: 30000 SR: Auto AGC: 150% AcT: 200 ms CS: >+2 IsM: Q IsW: 1.2 (MS) Frag.: sHCD NCE: 27,30,33			MS2 is performed on all ions with charge >+2. Fragmentation type is stepped HCD (sHCD). Each 1/3 of AGC will be separately treated with predefined increasing NCE (here 27, 30, 33). The ions are collected in the IRM. Once all portions were fragmented the whole batch is send to the OT for analysis at the same time. Advantage of this approach ... at low NCE the cross linker (DSSO) cleaves ... and releases the signature peptides .. at higher fragmentation these arms will be fragmented. So we have diagnostic peaks in the spectrum (which can be nicely used to identify CL spectra) and fragments from the arms for identification.

ACE_0716	Lumos	Tune v3.3.2782.28 Gradient: 70 & 105 min	Analyzer: FT Res.: 120000 SR: 375 - 1600 AGC: Standard AcT: Auto RF: 30 SF: -- DDM: CT/5sec	Analyzer: FT Res./ScR: 15000/- SR: Auto AGC: Standard AcT: 200ms CS: +3 to +7 IsM: Q IsW: 2 Frag.: sHCD NCE: 27, 30, 33			MS2 is performed on all ions with charge >+2. Fragmentation type is stepped HCD (sHCD). Each 1/3 of AGC will be separately treated with predefined increasing NCE (here 27, 30, 33). The ions are collected in the IRM. Once all portions were fragmented the whole batch is send to the OT for analysis at the same time. Advantage of this approach ... at low NCE the cross linker (DSSO) cleaves ... and releases the signature peptides .. at higher fragmentation these arms will be fragmented. So we have diagnostic peaks in the spectrum (which can be nicely used to identify CL spectra) and fragments from the arms for identification.
ACE_0630 MS2-MS2- MS3	Lumos	Tune v3.1.2412.25 Gradient: 60 min	Analyzer: FT Res.: 120000 SR: 375 - 1600 AGC: standard AcT: 50 RF: 30 SF: 10V DDM: CT/5sec	Analyzer: FT Res.: 30000 SR: Auto AGC: Auto AcT: 100 ms CS: >+2 IsM: Q IsW: 1.6 (MS) Frag.: CID NCE: 25	Analyzer: FT Res.: 50000 SR: Auto AGC: 400% AcT: 150 ms CS: >+2 IsM: Q IsW: 1.6 (MS) Frag.: sHCD NCE: 15, 30,50 NS: 1	Analyzer: IT Res.: 15000 SR: Auto AGC: 200% AcT: 120 ms CS: >+2 IsM: IT IsW: 2.5 (MS) 2 (MS2) Frag.: HCD NCE: 35 NS: 4	First MS2 is triggered by targeted mass difference 31.9721 (DSSO). Second MS2 is performed in OT with sHCD. Fragments from first MS2 with triggered mass difference were collected for MS3 experiments (HCD in IT). The whole sequence MS1-MS2-MS2/MS3 was repeated for 5 sec (cycle time between master scans).
ACE_0631 MS2	Lumos	Tune v v3.3.2782.28 Gradient: 67 min	Analyzer: FT Res.: 60000 SR: 400 - 1400 AGC: 150% AcT: auto RF: 30 SF: -- DDM: CT/3sec	Analyzer: FT Res.: 30000 SR: Auto AGC: 300% AcT: 100 ms CS: >+2 IsM: Q IsW: 1.6 (MS) Frag.: aHCD NCE: 25, 30			MS2 is performed on all ions with charge >+2. Fragmentation type is assisted HCD (aHCD). The MS selects the optimal NCE from provided list (here 25, 30). Intensity Threshold: 50000 Dynamic exclusion: 30 sec; ±10 ppm

Note: **FT**= Fourier Transform (Orbitrap); **IT**= Iontrap; **Q**= Quadrupol; **Res.**= max. Resolution at 200 m/z (Lumos) or 400 m/z (Elite) [FWHM (full width at half maximum)]; **SR**= scan range [m/z]; **AGC**= automatic gain control, max number of acquired ions per measurement; **AcT**= max. Ion acquisition time [ms]; **CS**= charge states used for fragmentation; **IsM**= Isolation mode (Q or IT), MS2 isolation and further is only done in IT; **IsW**= Isolation window [m/z], value followed by scan mode the isolation is based on (MS1, MS2 ...); **Frag.**= Fragmentation method; **HCD**= Higher-energy collisional dissociation; **CID**= Collision-induced dissociation; **ETD**= Electron-transfer dissociation; **ETHCD**= Electron-Transfer/Higher-Energy Collision Dissociation; **sHCD**= stepped HCD; **NCE**= normalized collision energy; **cycles**: number of MSn recorded or max cycle time; **RF**= RF Lens [%]; **SF**= Source Fragmentation [V]; **DDM**: Data dependent Mode (cycle time in seconds, CT/[s] or number of scans, NS); **NS**= Number of data dependent scans