Supporting Information

Selective neutral pH inhibitor of cathepsin B designed based on cleavage preferences at cytosolic and lysosomal pH conditions

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Supplemental Information

Table and Figures

Supplemental Table:

Table S1. Cathepsin B cleavages at positions 1-13 of peptide library substrates at 15 min. and 60 min. in MSP-MS analyses

Supplemental Figures:

Figure S1. Volcano plot of cathepsin B peptide cleavage products by MSP-MS at pH 7.2 and 4.6.
Cathepsin B cleavage products generated were quantitated by $\log_2$(Cat.B/inactivated enzyme) as a measure of fold-change of peptide intensities above the quenched inactive enzyme, and assessed for p values ($p < 0.05$).
(a) MSP-MS peptide products at pH 7.2.
(b) MSP-MS peptide products at pH 4.6.

Figure S2. MSP-MS assays: 0% False Positive Rate (FPR) for quenched cathepsin B control.

Figure S3. pH curves for the substrates Z-Arg-Lys-AMC, Z-Glu-Lys-AMC, and Z-Phe-Arg-AMC with absolute relative fluorescence (RFU) values for cathepsin B activity.

Figure S4. Selectivity of Z-Arg-Lys-AMC and Z-Glu-Lys-AMC substrates for cathepsin B compared to cathepsins L and V, at pH 7.2 and pH 4.6, with comparison to Z-Phe-Arg-AMC.
(a) Z-Arg-Lys-AMC substrate for cathepsin B compared to cathepsins L and V.
(b) Z-Glu-Lys-AMC substrate for cathepsin B compared to cathepsins L and V.
(c) Z-Phe-Arg-AMC substrate for cathepsin B compared to cathepsins L and V.

Figure S5. Chemical synthesis of Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibitors.
The chemical synthetic steps for Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibitors are illustrated. The AOMK warhead (blue), with the Lys residue (red), Arg or Glu at the P1 position (black), and the blocking group (purple) are shown. The chemical synthetic steps are described in the methods.

Figure S6. K_I determination of the cathepsin B inhibitors Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK.
(a) Z-Arg-Lys-AOMK K_I determination at pH 7.2 and pH 4.6.
(b) Z-Glu-Lys-AOMK K_I determination at pH 7.2 and pH 4.6.

Figure S7. Irreversible mechanism of Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibition.
(a) Z-Arg-Lys-AOMK inhibition.
(b) Z-Glu-Lys-AOMK inhibition
Inhibitors were evaluated for irreversible or reversible inhibition of cathepsin B by dilution experiments. Cathepsin B was pre-incubated with inhibitor at 10 times the IC_{50} concentration, followed by dilution to 1/10 the IC_{50} concentration, addition of substrate (Z-F-R-AMC), and monitoring activity in time-course assays. Inhibition of cathepsin B following dilution indicates the irreversible inhibitory mechanism of Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK.

Figure S8. Model of Z-Arg-Lys-AOMK inhibitor binding interactions to cathepsin B at pH 4.6.
(a) Model of the Z-Arg-Lys-AOMK inhibitor docking to cathepsin B at pH 4.6.
(b) Two-dimensional illustration of Z-Arg-Lys-AOMK and cathepsin B binding interactions at pH 4.6, showing lack of Glu245 interactions with the P2 Arg residue of the Z-Arg-Lys-AOMK inhibitor.

Figure S9. Model of Z-Glu-Lys-AOMK inhibitor binding interactions to cathepsin B at pH 4.6 and pH 7.2.
(a) pH 7.2 inhibitor docking. Two-dimensional illustration of Z-Glu-Lys-AMOK interactions with cathepsin B at pH 7.2.
(b) pH 4.6 inhibitor docking. Two-dimensional illustration of Z-Glu-Lys-AMOK interactions with cathepsin B at pH 4.6.

Figure S10. Z-Arg-Lys-AOMK inhibition of cathepsin B at pH 6.8 and IC_{50} value.

Supplemental Methods Information:

Methods S1. LC-MS/MS reports for cathepsin B at pH 7.2 and pH 4.6.
(a) MSP-MS analyses of Cathepsin B at pH 7.2 and pH 4.6.
(b) MSP-MS analyses of Cathepsin B with Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibitors at pH 7.2 & pH 4.6.

Methods S2. PEAKs reports for MS/MS data analyses.
(a) MSP-MS analyses of Cathepsin B at pH 7.2 and pH 4.6.
(b) MSP-MS analyses of Cathepsin B with Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibitors at pH 7.2 & pH 4.6.

Methods S3. Workbook of MSP-MS data

Table S1.

Cathepsin B cleavages at peptide bonds #1-13 of peptide library substrates at 15 min. and 60 min. in MSP-MS analyses

Cleavage at peptide bond number	pH 7.2 # cleavages		pH 4.6 # cleavages	
	15 min.	60 min.	15 min.	60 min.
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	1	1	0	1
8	0	2	1	2
9	1	2	1	2
10	5	11	11	30
11	1	2	3	8
12	30	44	67	95
13	2	3	1	4

Cathepsin B was subjected to MSP-MS (multiplex substrate profiling mass spectrometry) analysis at neutral pH 7.2 and acidic pH 4.6. The number of cleavages at each of the peptide bonds at positions 1-13 are illustrated for 15 minutes and 60 incubation of cathepsin B with the 228 14-mer peptide library in MSP-MS analyses. MSP-MS data by nano-LC-MS/MS was analyzed as described in the methods.

Figure S1. Volcano plot of cathepsin B peptide cleavage products by MSP-MS at pH 7.2 and 4.6. Cathepsin B cleavage products generated were quantitated by log_2(Cat.B/inactivated enzyme) as a measure of fold-change of peptide intensities above the quenched inactive enzyme, and assessed for p values ($p < 0.05$).
(a) MSP-MS peptide products at pH 7.2.
(b) MSP-MS peptide products at pH 4.6.

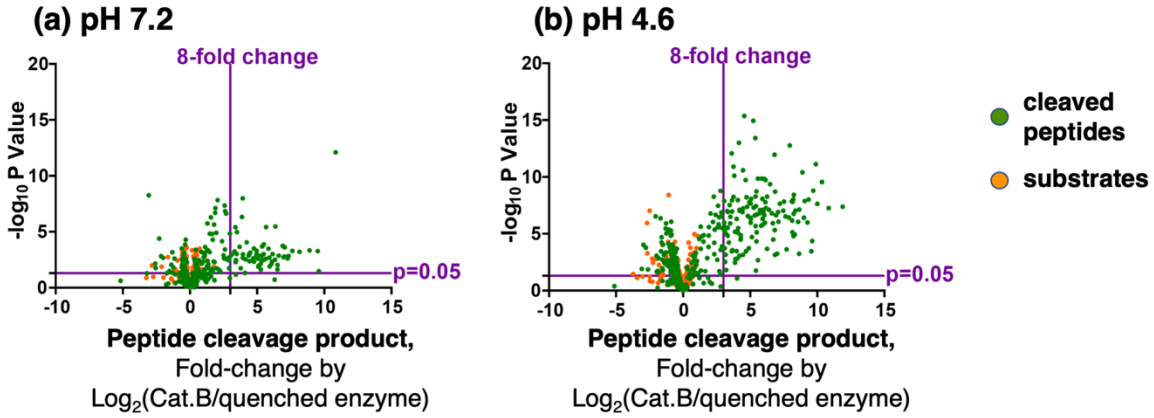

Figure S2. MSP-MS assays: 0% False Positive Rate (FPR) for quenched cathepsin B control. Analysis of the threshold for 0% false positive rate (FRP) was conducted by comparing quenched cathepsin B (inactivated by quenching in 8 M urea for 1 hr) incubated with the peptide library (used for MSP-MS) in two groups at pH 7.2 and pH 4.6, each group consisting of 4 replicates and subjected to LC-MS/MS analyses. Intensity values of peptides in these two groups was compared and plotted log_{10} of p values vs. log_2 fold change (comparison of the two groups). The criteria for 0% FPR was met with a threshold of 8-fold ratios of peptides in the two groups. Thus, 8-fold was in MSP-MS activity assays with cathepsin B for 0% FPR.

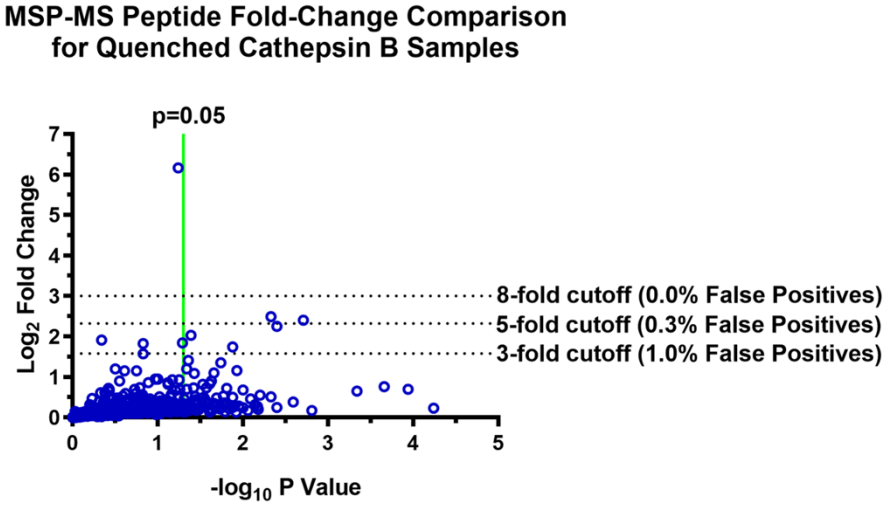

Figure S3. pH curves for the substrates Z-Arg-Lys-AMC, Z-Glu-Lys-AMC, and Z-Phe-Arg-AMC with absolute relative fluorescence (RFU) values for cathepsin B activity.

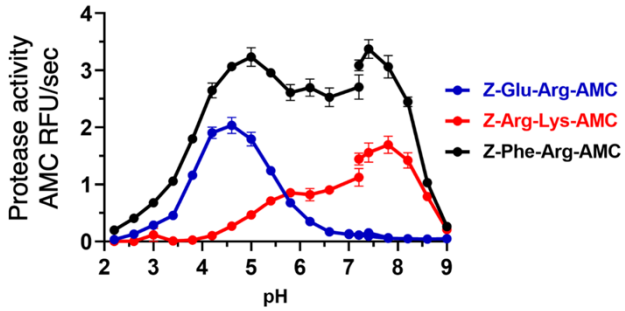

Figure S4. Selectivity of Z-Arg-Lys-AMC and Z-Glu-Lys-AMC substrates for cathepsin B compared to cathepsins L and V, at pH 7.2 and pH 4.6, with comparison to Z-Phe-Arg-AMC.
(a) Z-Arg-Lys-AMC substrate for cathepsin B compared to cathepsins L and V.
(b) Z-Glu-Lys-AMC substrate for cathepsin B compared to cathepsins L and V.
(c) Z-Phe-Arg-AMC substrate for cathepsin B compared to cathepsins L and V.

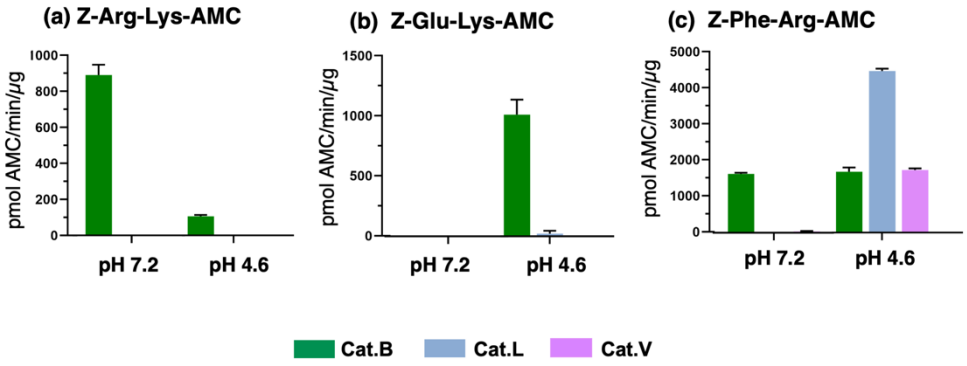

Figure S5. Chemical synthesis of Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibitors.
The chemical synthetic steps for Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibitors are illustrated. The AOMK warhead (blue), with the Lys residue (red), Arg or Glu at the P1 position (black), and the blocking group (purple) are shown. The chemical synthetic steps are described in the methods.

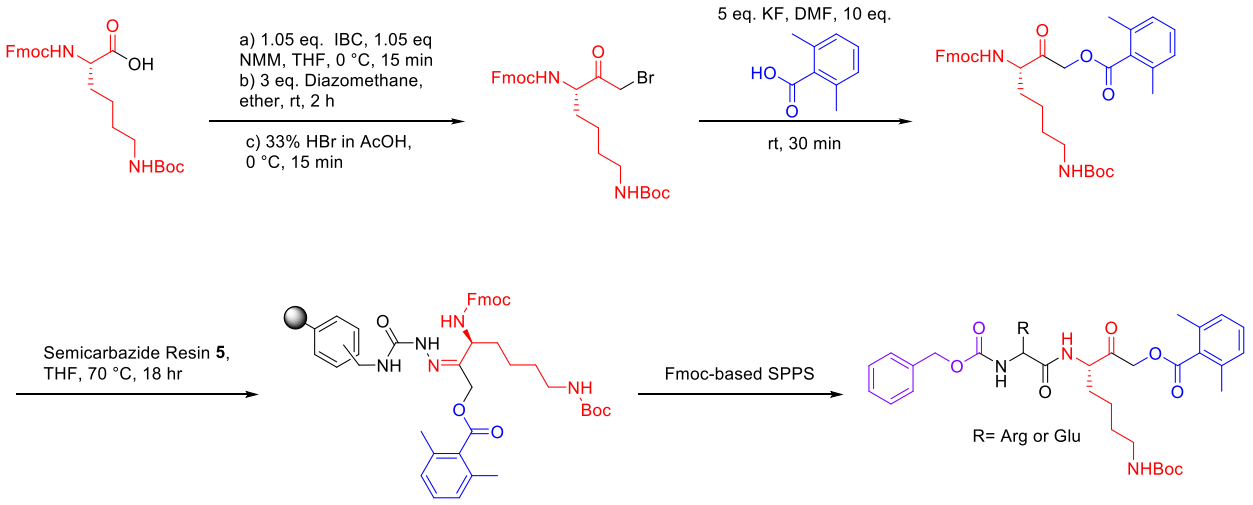

Figure S6. K_I determination of the cathepsin B inhibitors Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK.
(a) Z-Arg-Lys-AOMK K_I determination at pH 7.2 and pH 4.6.
(b) Z-Glu-Lys-AOMK K_I determination at pH 7.2 and pH 4.6.

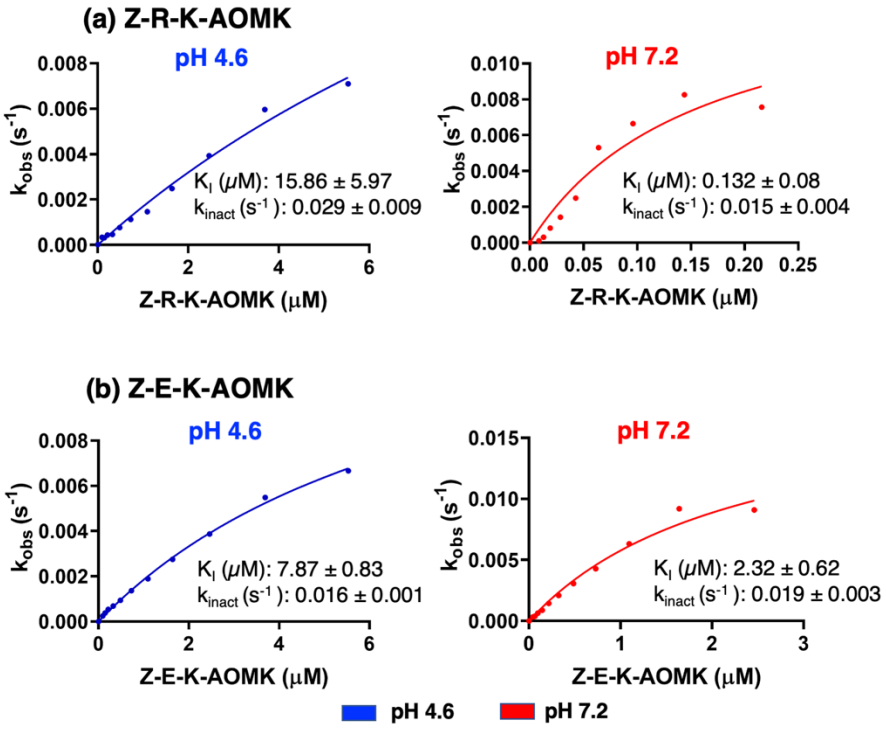

Figure S7. Irreversible mechanism of Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK inhibition.
(a) Z-Arg-Lys-AOMK inhibition.
(b) Z-Glu-Lys-AOMK inhibition
Inhibitors were evaluated for irreversible or reversible inhibition of cathepsin B by dilution experiments. Cathepsin B was pre-incubated with inhibitor at 10 times the IC_{50} concentration, followed by dilution to 1/10 the IC_{50} concentration, addition of substrate (Z-F-R-AMC), and monitoring activity in time-course assays. Inhibition of cathepsin B following dilution indicates the irreversible inhibitory mechanism of Z-Arg-Lys-AOMK and Z-Glu-Lys-AOMK.

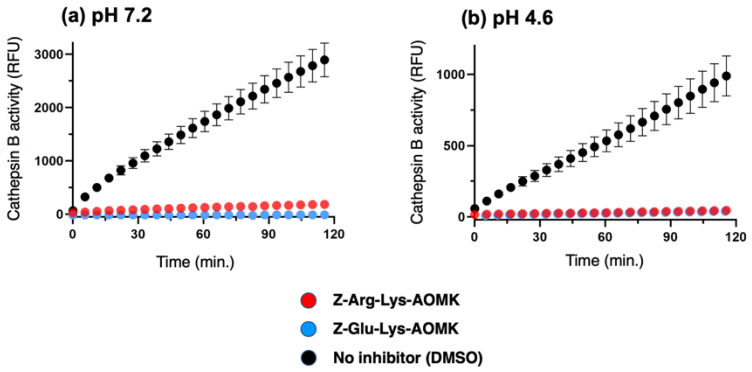

Figure S8. Model of Z-Arg-Lys-AOMK inhibitor binding interactions to cathepsin B at pH 4.6.
(a) Model of the Z-Arg-Lys-AOMK inhibitor docking to cathepsin B at pH 4.6.
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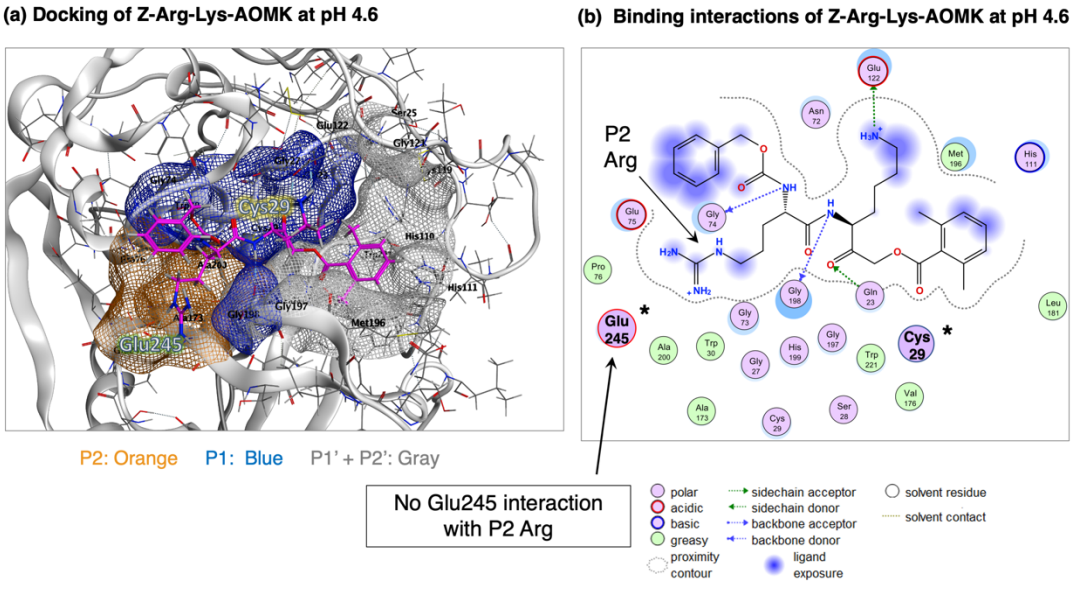


Figure S9. Model of Z-Glu-Lys-AOMK inhibitor binding interactions to cathepsin B at pH 4.6 and pH 7.2.

(a) pH 7.2 inhibitor docking. Two-dimensional illustration of Z-Glu-Lys-AMOK interactions with cathepsin B at pH 7.2.

(b) pH 4.6 inhibitor docking. Two-dimensional illustration of Z-Glu-Lys-AMOK interactions with cathepsin B at pH 4.6.

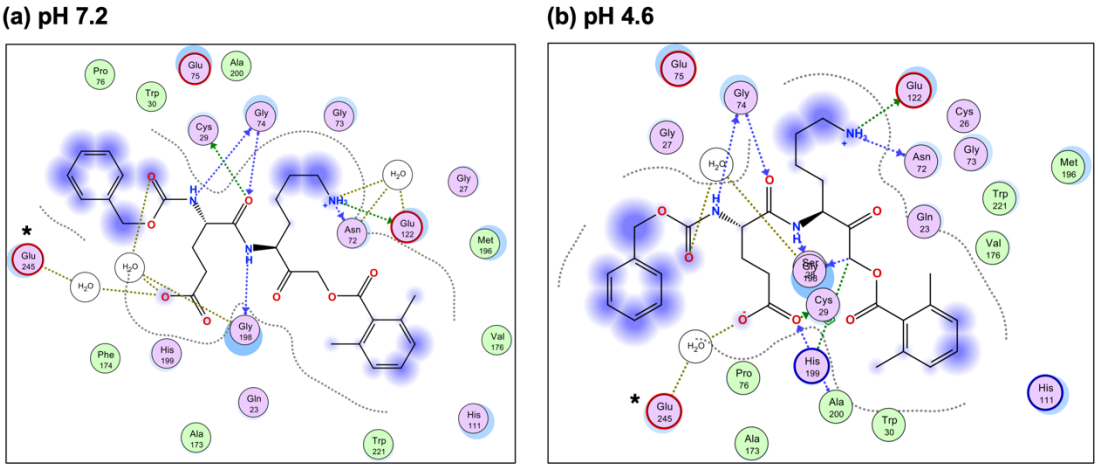
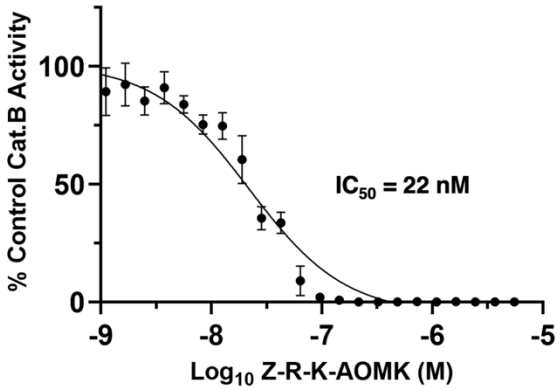


Figure S10. Z-Arg-Lys-AOMK inhibition of cathepsin B at pH 6.8 and IC_{50} value.



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Methods S3. Workbook of MSP-MS data

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Methods S3. Workbook of MSP-MS data

Supplement
Methods S1(a). LC-MS-MS Report, Cathepsin B MSP-MS at pH 4.6 and 7.2.

Samples were resuspended in 0.1% FA to a total peptide concentration of 114 µM
Each sample were injected once, 1 ul total volume per injection

The C18 column consisted of 1.7 µm bead size, 75 µm x 20 cm, heated to 65°C.

Solvent A – water, 0.1% formic acid
Solvent B – acetonitrile, 0.1% formic acid

Nano-LC gradient:

A	B	C	D
Time (min)	Flow (ul/min)	% solvent A	% solvent B
0	0.3	99	1
5	0.3	99	1
5.5	0.3	95	5
55	0.3	70	30
62	0.3	25	75
62.5	0.3	5	95
70	0.3	5	95
70.5	0.3	99	1

MS Sample List

MY_20180116_46_0000_1.raw	MY_20180116_46_0060_1.raw
MY_20180116_46_0000_2.raw	MY_20180116_46_0060_2.raw
MY_20180116_46_0000_3.raw	MY_20180116_46_0060_3.raw
MY_20180116_46_0000_4.raw	MY_20180116_46_0060_4.raw
MY_20180116_72_0000_1.raw	MY_20180116_72_0015_1.raw
MY_20180116_72_0000_2.raw	MY_20180116_72_0015_2.raw
MY_20180116_72_0000_3.raw	MY_20180116_72_0015_3.raw
MY_20180116_72_0000_4.raw	MY_20180116_72_0015_4.raw
MY_20180116_46_0015_1_Reinject.raw	MY_20180116_72_0060_1.raw
MY_20180116_46_0015_2.raw	MY_20180116_72_0060_2.raw
MY_20180116_46_0015_3.raw	MY_20180116_72_0060_3.raw
MY_20180116_46_0015_4.raw	MY_20180116_72_0060_4.raw

Q-Exactive MS Report:
Thermo Scientific SII for Xcalibur Method – Next Pages

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

---- Overview ----
Name: New Instrument Method
Comment:
Run time: 95.000 [min]
Instrument: Hook_nano on 2hyl8d2
Description:
---- Script ----
initial     Instrument Setup
            PumpModule.LoadingPump.%A.Equate: "%A"
            PumpModule.LoadingPump.%B.Equate: "%B"
            PumpModule.LoadingPump.%C.Equate: "%C"
            PumpModule.LoadingPump.Pressure.LowerLimit: 0 [bar]
            PumpModule.LoadingPump.Pressure.UpperLimit: 600 [bar]
            PumpModule.LoadingPump.MaximumFlowRampUp: 101 [µl/min²]
            PumpModule.LoadingPump.MaximumFlowRampDown: 10 [µl/min²]
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            PumpModule.NC_Pump.%B.Equate: "%B"
            PumpModule.NC_Pump.Pressure.LowerLimit: 0 [psi]
            PumpModule.NC_Pump.Pressure.UpperLimit: 9800 [psi]
            PumpModule.NC_Pump.MaximumFlowRampUp: 0.300 [µl/min²]
            PumpModule.NC_Pump.MaximumFlowRampDown: 0.300 [µl/min²]
            ColumnOven.TempCtrl: Off
            Sampler.LowDispersionMode: Off
            Sampler.WashSpeed: 4.000 [µl/s]
            Sampler.WashVolume: 50.000 [µl]
            Sampler.PunctureDepth: 7.000 [mm]
            Sampler.SampleHeight: 3.000 [mm]
            Sampler.WasteSpeed: 4.000 [µl/s]
            Sampler.DispenseDelay: 2.000 [s]
            Sampler.DispSpeed: 2.000 [µl/s]
            Sampler.DrawSpeed: 0.500 [µl/s]
            Sampler.DrawDelay: 5.000 [s]
            Sampler.RinseBetweenReinjections: No
            Sampler.InjectMode: UserProg
            Sampler.LoopWashFactor: 2.000
            Sampler.PumpDevice: "NC_Pump"
            Sampler.ReagentAVial: R1
            Sampler.ReagentBVial: R2
            Sampler.ReagentCVial: R3
            Sampler.ReagentDVial: R4
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            Sampler.ReagentFVial: RA1
            Sampler.ReagentGVial: RA1
            Sampler.ReagentHVial: RA1
            Sampler.TempCtrl: On
            Sampler.Temperature.Nominal: 7.0 [°C]
            Sampler.ReadyTempDelta: None
            Sampler.Temperature.LowerLimit: 4.0 [°C]
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Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

            Sampler.Temperature.UpperLimit: 45.0 [°C]
            Sampler.UdpSyringeValve Waste
            Sampler.UdpMixNeedleWash 100
            Sampler.UdpSyringeValve Needle
            Sampler.UdpDraw ReagentAVial,
                            (2*Sampler.NeedleVolume)+Sampler.LoopVolume,
                            GlobalSpeed,
                            4 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpInjectValve Load
            Sampler.UdpDraw SampleVial,
                            Sampler.Volume,
                            0.2 [µl/s],
                            3.000 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpDraw ReagentAVial,
                            (Sampler.LoopVolume-Sampler.Volume)*0.5+Sampler.NeedleVolume,
                            GlobalSpeed,
                            2 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpInjectValve Inject
            Sampler.UdpMixWait Sampler.LoopVolume/PumpModule.NC_Pump.Flow.Nominal*1.1*60
            Sampler.UdpInjectValve Load
            Sampler.UdpInjectMarker
            Sampler.UdpSyringeValve Waste
            Sampler.UdpMixNeedleWash 100.000 [µl]
0.000 [min] Inject Preparation
            Wait PumpModule.LoadingPump.Ready And PumpModule.NC_Pump.Ready And ColumnOven.Ready
0.000 [min] Inject
            Sampler.Inject
0.000 [min] Start Run
            PumpModule.NC_Pump.NC_Pump_Pressure.AcqOn
0.000 [min] Run
            PumpModule.LoadingPump.Flow.Nominal: 0.000 [µl/min]
            PumpModule.LoadingPump.%B.Value: 0.0 [%]
            PumpModule.LoadingPump.%C.Value: 0.0 [%]
            PumpModule.LoadingPump.Curve: 5
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 1.0 [%]
            PumpModule.NC_Pump.Curve: 5
0.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 5.0 [%]
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20.000 [min]
            PumpModule.LoadingPump.Flow.Nominal: 0.000 [µl/min]
            PumpModule.LoadingPump.%B.Value: 0.0 [%]
            PumpModule.LoadingPump.%C.Value: 0.0 [%]
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Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

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Thermo Scientific SII for Xcalibur Method

            PumpModule.LoadingPump.Curve: 5
60.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 30.0 [%]
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65.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 85.0 [%]
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75.000 [min]
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85.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.400 [µl/min]
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85.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 1.0 [%]
            PumpModule.NC_Pump.Curve: 5
95.000 [min] Stop Run
            PumpModule.NC_Pump.NC_Pump_Pressure.AcqOff
```

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

Method of Q Exactive

Overall method settings

Global Settings	
Use lock masses	off
Lock mass injection	–
Chrom. peak width (FWHM)	15 s
Time	
Method duration	95.00 min
Customized Tolerances (+/-)	
Lock Masses	–
Inclusion	–
Exclusion	–
Neutral Loss	–
Mass Tags	–
Dynamic Exclusion	10.0 ppm

Experiment

Full MS / dd-MS² (TopN)

General	
Runtime	0 to 95 min
Polarity	Positive
In-source CID	0.0 eV
Default charge state	2
Inclusion	–
Exclusion	–
Tags	–
Full MS	
Microscans	1
Resolution	70,000
AGC target	3e6
Maximum IT	100 ms
Number of scan ranges	1
Scan range	250 to 1500 m/z
Spectrum data type	Profile
dd-MS² / dd-SIM	
Microscans	1
Resolution	17,500
AGC target	1e5
Maximum IT	50 ms
Loop count	12
MSX count	1
TopN	12
Isolation window	1.5 m/z
Isolation offset	0.0 m/z
Scan range	200 to 2000 m/z
Fixed first mass	150.0 m/z

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

(N)CE / stepped (N)CE	nce: 28
Spectrum data type	Centroid
dd Settings	
Minimum AGC target	3.00e2
Intensity threshold	6.0e3
Apex trigger	–
Charge exclusion	unassigned, 1
Peptide match	Preferred
Exclude isotopes	on
Dynamic exclusion	20.0 s
If idle ..	do not pick others

Setup

Tunefiles
General
Switch Count 0
Base Tunefile C:\Xcalibur\methods\CL_nanoTune_20180409.mstune

Contact Closure
General

Used	False
Start in Closed	True
Switch Count	0

Syringe
General

Used	False	
Start in OFF	True	
Stop at end of run	False	
Switch Count	0	
Pump setup		
Syringe type	Hamilton	
Flow rate	3.000	µL/min
Inner diameter	2.303	mm
Volume	250	µL

Divert Valve A
General

Used	False
Start in 1-2	True
Switch Count	0

Divert Valve B
General

Used	False
Start in 1-2	True
Switch Count	0

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

---- Overview ----
Name: New Instrument Method
Comment:
Run time: 95.000 [min]
Instrument: Hook_nano on 2hyl8d2
Description:
---- Script ----
initial     Instrument Setup
            PumpModule.LoadingPump.%A.Equate: "%A"
            PumpModule.LoadingPump.%B.Equate: "%B"
            PumpModule.LoadingPump.%C.Equate: "%C"
            PumpModule.LoadingPump.Pressure.LowerLimit: 0 [bar]
            PumpModule.LoadingPump.Pressure.UpperLimit: 600 [bar]
            PumpModule.LoadingPump.MaximumFlowRampUp: 101 [µl/min²]
            PumpModule.LoadingPump.MaximumFlowRampDown: 10 [µl/min²]
            PumpModule.NC_Pump.%A.Equate: "%A"
            PumpModule.NC_Pump.%B.Equate: "%B"
            PumpModule.NC_Pump.Pressure.LowerLimit: 0 [psi]
            PumpModule.NC_Pump.Pressure.UpperLimit: 9800 [psi]
            PumpModule.NC_Pump.MaximumFlowRampUp: 0.300 [µl/min²]
            PumpModule.NC_Pump.MaximumFlowRampDown: 0.300 [µl/min²]
            ColumnOven.TempCtrl: Off
            Sampler.LowDispersionMode: Off
            Sampler.WashSpeed: 4.000 [µl/s]
            Sampler.WashVolume: 50.000 [µl]
            Sampler.PunctureDepth: 7.000 [mm]
            Sampler.SampleHeight: 3.000 [mm]
            Sampler.WasteSpeed: 4.000 [µl/s]
            Sampler.DispenseDelay: 2.000 [s]
            Sampler.DispSpeed: 2.000 [µl/s]
            Sampler.DrawSpeed: 0.500 [µl/s]
            Sampler.DrawDelay: 5.000 [s]
            Sampler.RinseBetweenReinjections: No
            Sampler.InjectMode: UserProg
            Sampler.LoopWashFactor: 2.000
            Sampler.PumpDevice: "NC_Pump"
            Sampler.ReagentAVial: R1
            Sampler.ReagentBVial: R2
            Sampler.ReagentCVial: R3
            Sampler.ReagentDVial: R4
            Sampler.ReagentEVial: RA1
            Sampler.ReagentFVial: RA1
            Sampler.ReagentGVial: RA1
            Sampler.ReagentHVial: RA1
            Sampler.TempCtrl: On
            Sampler.Temperature.Nominal: 7.0 [°C]
            Sampler.ReadyTempDelta: None
            Sampler.Temperature.LowerLimit: 4.0 [°C]
```

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

            Sampler.Temperature.UpperLimit: 45.0 [°C]
            Sampler.UdpSyringeValve Waste
            Sampler.UdpMixNeedleWash 100
            Sampler.UdpSyringeValve Needle
            Sampler.UdpDraw ReagentAVial,
                            (2*Sampler.NeedleVolume)+Sampler.LoopVolume,
                            GlobalSpeed,
                            4 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpInjectValve Load
            Sampler.UdpDraw SampleVial,
                            Sampler.Volume,
                            0.2 [µl/s],
                            3.000 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpDraw ReagentAVial,
                            (Sampler.LoopVolume-Sampler.Volume)*0.5+Sampler.NeedleVolume,
                            GlobalSpeed,
                            2 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpInjectValve Inject
            Sampler.UdpMixWait Sampler.LoopVolume/PumpModule.NC_Pump.Flow.Nominal*1.1*60
            Sampler.UdpInjectValve Load
            Sampler.UdpInjectMarker
            Sampler.UdpSyringeValve Waste
            Sampler.UdpMixNeedleWash 100.000 [µl]
0.000 [min] Inject Preparation
            Wait PumpModule.LoadingPump.Ready And PumpModule.NC_Pump.Ready And ColumnOven.Ready
0.000 [min] Inject
            Sampler.Inject
0.000 [min] Start Run
            PumpModule.NC_Pump.NC_Pump_Pressure.AcqOn
0.000 [min] Run
            PumpModule.LoadingPump.Flow.Nominal: 0.000 [µl/min]
            PumpModule.LoadingPump.%B.Value: 0.0 [%]
            PumpModule.LoadingPump.%C.Value: 0.0 [%]
            PumpModule.LoadingPump.Curve: 5
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 1.0 [%]
            PumpModule.NC_Pump.Curve: 5
0.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 5.0 [%]
            PumpModule.NC_Pump.Curve: 5
20.000 [min]
            PumpModule.LoadingPump.Flow.Nominal: 0.000 [µl/min]
            PumpModule.LoadingPump.%B.Value: 0.0 [%]
            PumpModule.LoadingPump.%C.Value: 0.0 [%]
```

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

            PumpModule.LoadingPump.Curve: 5
60.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 30.0 [%]
            PumpModule.NC_Pump.Curve: 5
65.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 85.0 [%]
            PumpModule.NC_Pump.Curve: 5
75.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 85.0 [%]
            PumpModule.NC_Pump.Curve: 5
75.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.400 [µl/min]
            PumpModule.NC_Pump.%B.Value: 90.0 [%]
            PumpModule.NC_Pump.Curve: 5
85.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.400 [µl/min]
            PumpModule.NC_Pump.%B.Value: 90.0 [%]
            PumpModule.NC_Pump.Curve: 5
85.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 1.0 [%]
            PumpModule.NC_Pump.Curve: 5
95.000 [min] Stop Run
            PumpModule.NC_Pump.NC_Pump_Pressure.AcqOff
```

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

Method of Q Exactive

Overall method settings

Global Settings		
Use lock masses	off	
Lock mass injection	–	
Chrom. peak width (FWHM)	15	s
Time		
Method duration	95.00	min
Customized Tolerances (+/-)		
Lock Masses	–	
Inclusion	–	
Exclusion	–	
Neutral Loss	–	
Mass Tags	–	
Dynamic Exclusion	10.0	ppm

Experiment

Full MS / dd-MS² (TopN)

General		
Runtime	0 to 95	min
Polarity	Positive	
In-source CID	0.0	eV
Default charge state	2	
Inclusion	–	
Exclusion	–	
Tags	–	
Full MS		
Microscans	1	
Resolution	70,000	
AGC target	3e6	
Maximum IT	100	ms
Number of scan ranges	1	
Scan range	250 to 1500	m/z
Spectrum data type	Profile	
dd-MS² / dd-SIM		
Microscans	1	
Resolution	17,500	
AGC target	1e5	
Maximum IT	50	ms
Loop count	12	
MSX count	1	
TopN	12	
Isolation window	1.5	m/z
Isolation offset	0.0	m/z
Scan range	200 to 2000	m/z
Fixed first mass	150.0	m/z

Instrument Method: ZJ_MSP_MS_20180717_25cm_5uL

(N)CE / stepped (N)CE	nce: 28
Spectrum data type	Centroid
dd Settings	
Minimum AGC target	3.00e2
Intensity threshold	6.0e3
Apex trigger	–
Charge exclusion	unassigned, 1
Peptide match	Preferred
Exclude isotopes	on
Dynamic exclusion	20.0 s
If idle ..	do not pick others

Setup

Tunefiles

General

Switch Count	0
Base Tunefile	C:\Xcalibur\methods\CL_nanoTune_20180409.mstune

Contact Closure

General

Used	False
Start in Closed	True
Switch Count	0

Syringe

General

Used	False
Start in OFF	True
Stop at end of run	False
Switch Count	0

Pump setup

Syringe type	Hamilton	
Flow rate	3.000	µL/min
Inner diameter	2.303	mm
Volume	250	µL

Divert Valve A

General

Used	False
Start in 1-2	True
Switch Count	0

Divert Valve B

General

Used	False
Start in 1-2	True
Switch Count	0

Supplement
Methods S1(b). LC-MS-MS Report, Cathepsin B MSP-MS with Peptidic AOMK Inhibitors, pH 4.6 and 7.2.

Samples were resuspended in 0.1% TFA to a total peptide concentration of 28.5 µM
Each sample were injected once, 4 ul total volume per injection

The C18 column consisted of 1.7 µm bead size, 75 µm x 20 cm, heated to 65°C.

Solvent A – water, 0.1% formic acid
Solvent B – acetonitrile, 0.1% formic acid

Nano-LC gradient:

A	B	C	D
Time (min)	Flow (ul/min)	% solvent A	% solvent B
0	0.3	99	1
0.1	0.3	95	5
60	0.3	70	30
65	0.3	15	85
75	0.3	15	85
75.1	0.4	10	90
85	0.4	10	90
85.1	0.3	99	1

MS Sample List

7_RK_d_MY_pH72_60.raw	4_RK_d_MY_pH46_60.raw
7_RK_c_MY_pH72_60.raw	4_RK_c_MY_pH46_60.raw
7_RK_b_MY_pH72_60.raw	4_RK_b_MY_pH46_60_Reinject.raw
7_RK_a_MY_pH72_60.raw	4_RK_a_MY_pH46_60.raw
7_Q_d_MY_pH72_60.raw	4_Q_d_MY_pH46_60.raw
7_Q_c_MY_pH72_60.raw	4_Q_c_MY_pH46_60.raw
7_Q_b_MY_pH72_60.raw	4_Q_b_MY_pH46_60.raw
7_Q_a_MY_pH72_60.raw	4_Q_a_MY_pH46_60.raw
7_EK_d_MY_pH72_60.raw	4_EK_d_MY_pH46_60.raw
7_EK_c_MY_pH72_60.raw	4_EK_c_MY_pH46_60.raw
7_EK_b_MY_pH72_60.raw	4_EK_b_MY_pH46_60.raw
7_EK_a_MY_pH72_60.raw	4_EK_a_MY_pH46_60.raw
7_DMSO_d_MY_pH72_60.raw	4_DMSO_d_MY_pH46_60.raw
7_DMSO_c_MY_pH72_60.raw	4_DMSO_c_MY_pH46_60.raw
7_DMSO_b_MY_pH72_60.raw	4_DMSO_b_MY_pH46_60.raw
7_DMSO_a_MY_pH72_60.raw	4_DMSO_a_MY_pH46_60.raw

Q-Exactive MS Report:
Thermo Scientific SII for Xcalibur Method – Next Pages

Instrument Method: ZJ_MSP_MS_20200323_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

---- Overview ----
Name: New Instrument Method
Comment:
Run time: 100.000 [min]
Instrument: Hook_nano on 2hyl8d2
Description:
---- Script ----
initial     Instrument Setup
            PumpModule.LoadingPump.%A.Equate: "%A"
            PumpModule.LoadingPump.%B.Equate: "%B"
            PumpModule.LoadingPump.%C.Equate: "%C"
            PumpModule.LoadingPump.Pressure.LowerLimit: 0 [bar]
            PumpModule.LoadingPump.Pressure.UpperLimit: 600 [bar]
            PumpModule.LoadingPump.MaximumFlowRampUp: 101 [µl/min²]
            PumpModule.LoadingPump.MaximumFlowRampDown: 10 [µl/min²]
            PumpModule.NC_Pump.%A.Equate: "%A"
            PumpModule.NC_Pump.%B.Equate: "%B"
            PumpModule.NC_Pump.Pressure.LowerLimit: 0 [psi]
            PumpModule.NC_Pump.Pressure.UpperLimit: 9800 [psi]
            PumpModule.NC_Pump.MaximumFlowRampUp: 0.300 [µl/min²]
            PumpModule.NC_Pump.MaximumFlowRampDown: 0.300 [µl/min²]
            ColumnOven.TempCtrl: Off
            Sampler.LowDispersionMode: Off
            Sampler.WashSpeed: 4.000 [µl/s]
            Sampler.WashVolume: 50.000 [µl]
            Sampler.PunctureDepth: 7.000 [mm]
            Sampler.SampleHeight: 3.000 [mm]
            Sampler.WasteSpeed: 4.000 [µl/s]
            Sampler.DispenseDelay: 2.000 [s]
            Sampler.DispSpeed: 2.000 [µl/s]
            Sampler.DrawSpeed: 0.500 [µl/s]
            Sampler.DrawDelay: 5.000 [s]
            Sampler.RinseBetweenReinjections: No
            Sampler.InjectMode: UserProg
            Sampler.LoopWashFactor: 2.000
            Sampler.PumpDevice: "NC_Pump"
            Sampler.ReagentAVial: R1
            Sampler.ReagentBVial: R2
            Sampler.ReagentCVial: R3
            Sampler.ReagentDVial: R4
            Sampler.ReagentEVial: RA1
            Sampler.ReagentFVial: RA1
            Sampler.ReagentGVial: RA1
            Sampler.ReagentHVial: RA1
            Sampler.TempCtrl: On
            Sampler.Temperature.Nominal: 7.0 [°C]
            Sampler.ReadyTempDelta: None
            Sampler.Temperature.LowerLimit: 4.0 [°C]
```

Instrument Method: ZJ_MSP_MS_20200323_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

            Sampler.Temperature.UpperLimit: 45.0 [°C]
            Sampler.UdpSyringeValve Waste
            Sampler.UdpMixNeedleWash 100
            Sampler.UdpSyringeValve Needle
            Sampler.UdpDraw ReagentAVial,
                            (2*Sampler.NeedleVolume)+Sampler.LoopVolume,
                            GlobalSpeed,
                            4 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpInjectValve Load
            Sampler.UdpDraw SampleVial,
                            Sampler.Volume,
                            0.2 [µl/s],
                            3.000 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpDraw ReagentAVial,
                            (Sampler.LoopVolume-Sampler.Volume)*0.5+Sampler.NeedleVolume,
                            GlobalSpeed,
                            2 [mm]
            Sampler.UdpMixWait 4 [s]
            Sampler.UdpInjectValve Inject
            Sampler.UdpMixWait Sampler.LoopVolume/PumpModule.NC_Pump.Flow.Nominal*1.1*60
            Sampler.UdpInjectValve Load
            Sampler.UdpInjectMarker
            Sampler.UdpSyringeValve Waste
            Sampler.UdpMixNeedleWash 100.000 [µl]
0.000 [min] Inject Preparation
            Wait PumpModule.LoadingPump.Ready And PumpModule.NC_Pump.Ready And ColumnOven.Ready
0.000 [min] Inject
            Sampler.Inject
0.000 [min] Start Run
            PumpModule.NC_Pump.NC_Pump_Pressure.AcqOn
0.000 [min] Run
            PumpModule.LoadingPump.Flow.Nominal: 0.000 [µl/min]
            PumpModule.LoadingPump.%B.Value: 0.0 [%]
            PumpModule.LoadingPump.%C.Value: 0.0 [%]
            PumpModule.LoadingPump.Curve: 5
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 1.0 [%]
            PumpModule.NC_Pump.Curve: 5
0.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 5.0 [%]
            PumpModule.NC_Pump.Curve: 5
20.000 [min]
            PumpModule.LoadingPump.Flow.Nominal: 0.000 [µl/min]
            PumpModule.LoadingPump.%B.Value: 0.0 [%]
            PumpModule.LoadingPump.%C.Value: 0.0 [%]
```

Instrument Method: ZJ_MSP_MS_20200323_25cm_5uL

```
Thermo Scientific SII for Xcalibur Method

            PumpModule.LoadingPump.Curve: 5
65.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 40.0 [%]
            PumpModule.NC_Pump.Curve: 5
70.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 85.0 [%]
            PumpModule.NC_Pump.Curve: 5
80.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 85.0 [%]
            PumpModule.NC_Pump.Curve: 5
80.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.400 [µl/min]
            PumpModule.NC_Pump.%B.Value: 90.0 [%]
            PumpModule.NC_Pump.Curve: 5
90.000 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.400 [µl/min]
            PumpModule.NC_Pump.%B.Value: 90.0 [%]
            PumpModule.NC_Pump.Curve: 5
90.100 [min]
            PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
            PumpModule.NC_Pump.%B.Value: 1.0 [%]
            PumpModule.NC_Pump.Curve: 5
100.000 [min] Stop Run
            PumpModule.NC_Pump.NC_Pump_Pressure.AcqOff
```

Instrument Method: ZJ_MSP_MS_20200323_25cm_5uL

Method of Q Exactive

Overall method settings

Global Settings	
Use lock masses	off
Lock mass injection	–
Chrom. peak width (FWHM)	15 s
Time	
Method duration	95.00 min
Customized Tolerances (+/-)	
Lock Masses	–
Inclusion	–
Exclusion	–
Neutral Loss	–
Mass Tags	–
Dynamic Exclusion	10.0 ppm

Experiment

Full MS / dd-MS² (TopN)

General	
Runtime	0 to 95 min
Polarity	Positive
In-source CID	0.0 eV
Default charge state	2
Inclusion	–
Exclusion	–
Tags	–
Full MS	
Microscans	1
Resolution	70,000
AGC target	3e6
Maximum IT	100 ms
Number of scan ranges	1
Scan range	250 to 1500 m/z
Spectrum data type	Profile
dd-MS² / dd-SIM	
Microscans	1
Resolution	17,500
AGC target	1e5
Maximum IT	50 ms
Loop count	15
MSX count	1
TopN	15
Isolation window	1.5 m/z
Isolation offset	0.0 m/z
Scan range	200 to 2000 m/z
Fixed first mass	150.0 m/z

Instrument Method: ZJ_MSP_MS_20200323_25cm_5uL

(N)CE / stepped (N)CE	nce: 28
Spectrum data type	Centroid
dd Settings	
Minimum AGC target	3.00e2
Intensity threshold	6.0e3
Apex trigger	–
Charge exclusion	unassigned, 1
Peptide match	Preferred
Exclude isotopes	on
Dynamic exclusion	20.0 s
If idle ..	do not pick others

Setup

Tunefiles

General

Switch Count	0
Base Tunefile	C:\Xcalibur\methods\ZJ_nanoTune_20200115.mstune

Contact Closure

General

Used	False
Start in Closed	True
Switch Count	0

Syringe

General

Used	False
Start in OFF	True
Stop at end of run	False
Switch Count	0

Pump setup

Syringe type	Hamilton	
Flow rate	3.000	µL/min
Inner diameter	2.303	mm
Volume	250	µL

Divert Valve A

General

Used	False
Start in 1-2	True
Switch Count	0

Divert Valve B

General

Used	False
Start in 1-2	True
Switch Count	0

Summary

1. Notes

2. Result Statistics

Figure 1. False discovery rate (FDR) curve. X axis is the number of peptide-spectrum matches (PSM) being kept. Y axis is the corresponding FDR.

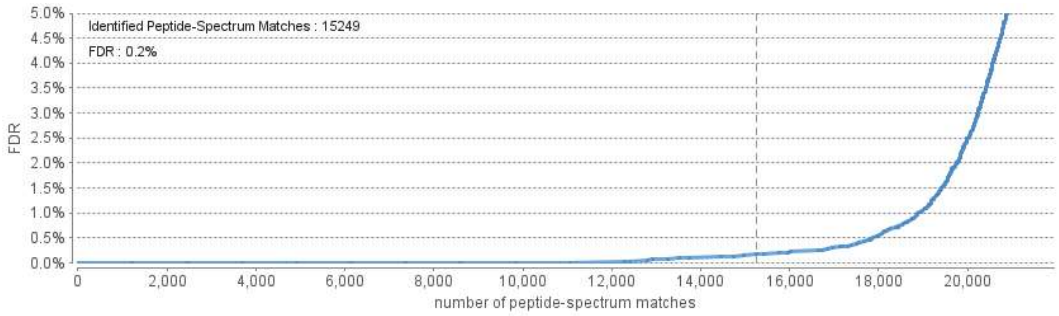

Figure 2. PSM score distribution. **(a)** Distribution of PEAKS peptide score; **(b)** Scatterplot of PEAKS peptide score versus precursor mass error.

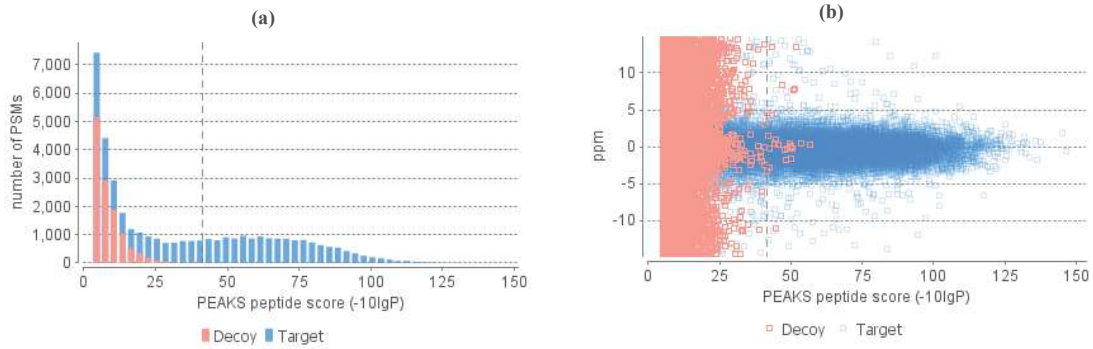

Figure 3. De novo result validation. Distribution of residue local confidence: **(a)** Residues in de novo sequences validated by confident database peptide assignment; **(b)** Residues in "de novo only" sequences.

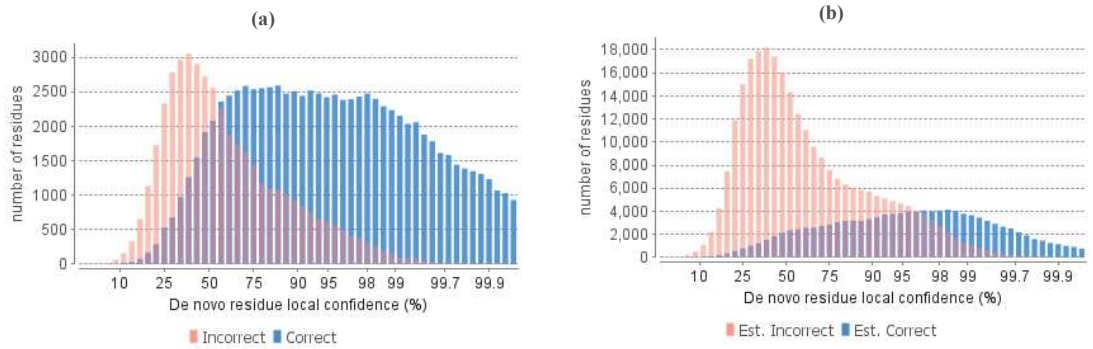

Table 1. Statistics of data.

# of MS scans	224271
# of MS/MS scans	332306

Table 2. Result filtration parameters.

Peptide -10lgP	≥41.5
Peptide Ascore	≥0
Protein -10lgP	≥89.5
Proteins unique peptides	≥0
De novo ALC Score	≥50%

Table 3. Statistics of filtered result.

Table 4. PTM profile.

Name	ΔMass	Position	#PSM	-10lgP	Area	AScore

Peptide-Spectrum Matches	15249
Peptide sequences	740
Protein groups	199
Proteins	199
Proteins (#Unique Peptides)	151 (>2); 37 (=2); 9 (=1);
FDR (Peptide-Spectrum Matches)	0.2%
FDR (Peptide Sequences)	0.9%
FDR (Protein)	1.0%
De Novo Only Spectra	27127

3. Experiment Control

Figure 4. Precursor mass error of peptide-spectrum matches (PSM) in filtered result. **(a)** Distribution of precursor mass error in ppm; **(b)** Scatterplot of precursor m/z versus precursor mass error in ppm.

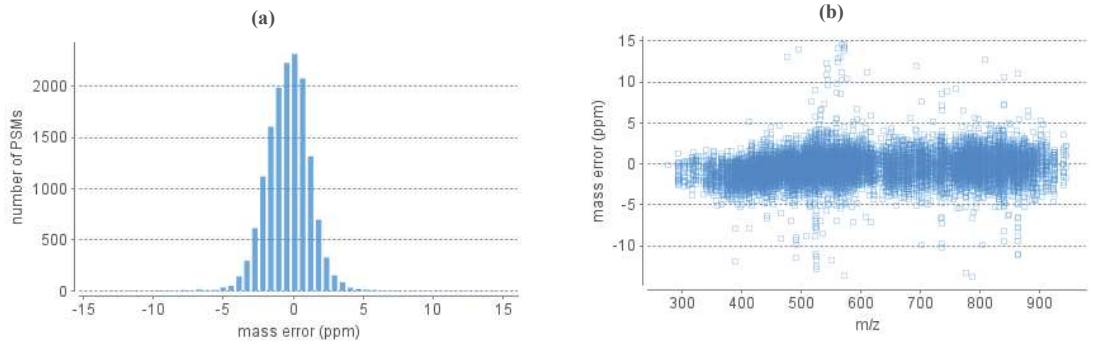

Table 5. Number of identified peptides in each sample by the number of missed cleavages

Missed Cleavages	0	1	2	3	4+
46_0000_1	0	0	0	0	5
46_0000_2	0	0	0	0	27
46_0000_3	0	0	0	0	34
46_0000_4	0	0	0	0	21
46_0015_1	0	0	0	0	62
46_0015_2	0	0	0	0	49
46_0015_3	0	0	0	0	14
46_0015_4	0	0	0	0	2
46_0060_1	0	0	0	0	5
46_0060_2	0	0	0	0	59
...					

4. Other Information

Table 6. Search parameters.

Search Engine Name: PEAKS
Parent Mass Error Tolerance: 15.0 ppm
Fragment Mass Error Tolerance: 0.01 Da
Precursor Mass Search Type: monoisotopic
Enzyme: None
Max Missed Cleavages: 100
Non-specific Cleavage: both
Max Variable PTM Per Peptide: 3
Database: TDP_237library_07202017
Taxon: All
Searched Entry: 228
FDR Estimation: Enabled
Different data refine parameters are used for this search:

Table 7. Instrument parameters.

Fractions: MY_20180116_46_0000_1.raw, MY_20180116_46_0000_2.raw, MY_20180116_46_0000_3.raw, MY_20180116_46_0000_4.raw, MY_20180116_46_0015_1_Reinject.raw, MY_20180116_46_0015_2.raw, MY_20180116_46_0015_3.raw, MY_20180116_46_0015_4.raw, MY_20180116_46_0060_1.raw, MY_20180116_46_0060_2.raw, MY_20180116_46_0060_3.raw, MY_20180116_46_0060_4.raw, MY_20180116_72_0000_1.raw, MY_20180116_72_0000_2.raw, MY_20180116_72_0000_3.raw, MY_20180116_72_0000_4.raw, MY_20180116_72_0015_1.raw, MY_20180116_72_0015_2.raw, MY_20180116_72_0015_3.raw, MY_20180116_72_0015_4.raw, MY_20180116_72_0060_1.raw, MY_20180116_72_0060_2.raw, MY_20180116_72_0060_3.raw, MY_20180116_72_0060_4.raw
Ion Source: ESI(nano-spray)
Fragmentation Mode: high energy CID (y and b ions)
MS Scan Mode: FT-ICR/Orbitrap
MS/MS Scan Mode: FT-ICR/Orbitrap

1. Notes

2. Result Statistics

Figure 1. False discovery rate (FDR) curve. X axis is the number of peptide-spectrum matches (PSM) being kept. Y axis is the corresponding FDR.

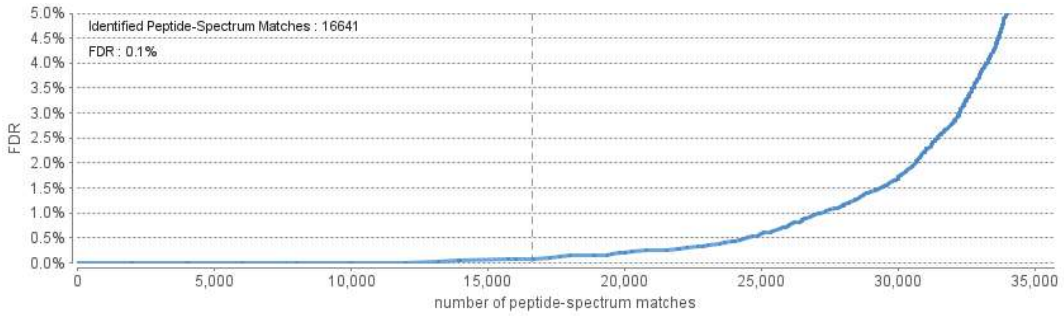

Figure 2. PSM score distribution. **(a)** Distribution of PEAKS peptide score; **(b)** Scatterplot of PEAKS peptide score versus precursor mass error.

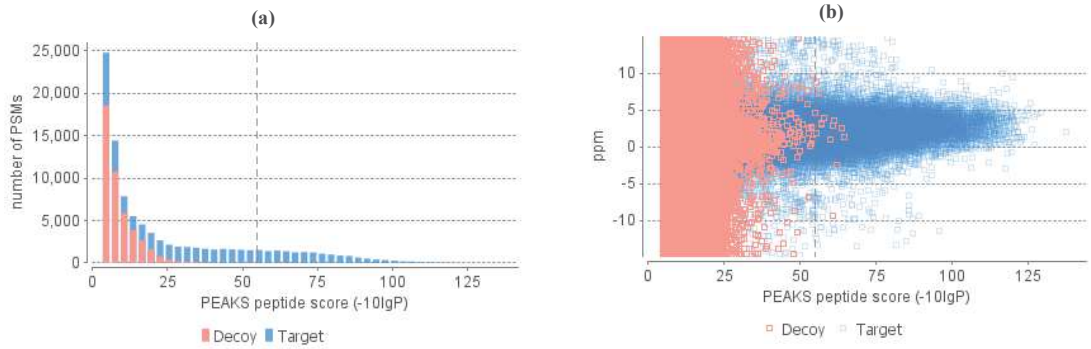

Figure 3. De novo result validation. Distribution of residue local confidence: **(a)** Residues in de novo sequences validated by confident database peptide assignment; **(b)** Residues in "de novo only" sequences.

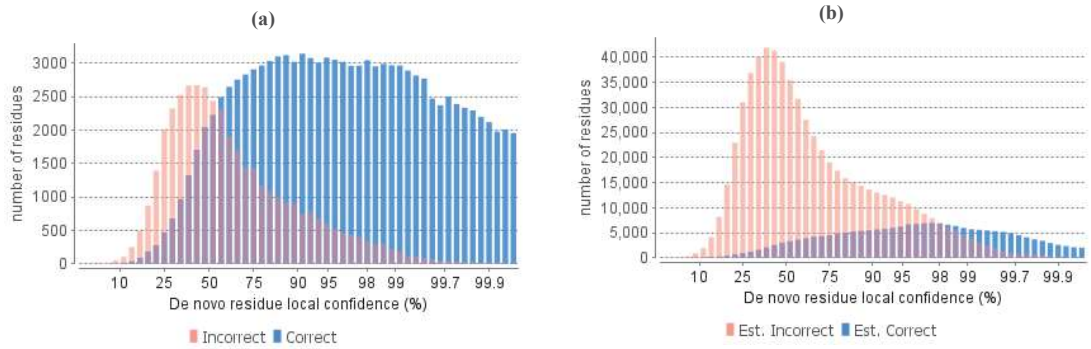

Table 1. Statistics of data.

# of MS scans	462226
# of MS/MS scans	995943

Table 2. Result filtration parameters.

Peptide -10lgP	≥55
Peptide Ascore	≥0
Protein -10lgP	≥20
Proteins unique peptides	≥0
De novo ALC Score	≥50%

Table 3. Statistics of filtered result.

Table 4. PTM profile.

Name	ΔMass	Position	#PSM	-10lgP	Area	AScore

Peptide-Spectrum Matches	16641
Peptide sequences	639
Protein groups	435
Proteins	435
Proteins (#Unique Peptides)	109 (>2); 34 (=2); 32 (=1);
FDR (Peptide-Spectrum Matches)	0.1%
FDR (Peptide Sequences)	0.9%
FDR (Protein)	106.2%
De Novo Only Spectra	69052

3. Experiment Control

Figure 4. Precursor mass error of peptide-spectrum matches (PSM) in filtered result. **(a)** Distribution of precursor mass error in ppm; **(b)** Scatterplot of precursor m/z versus precursor mass error in ppm.

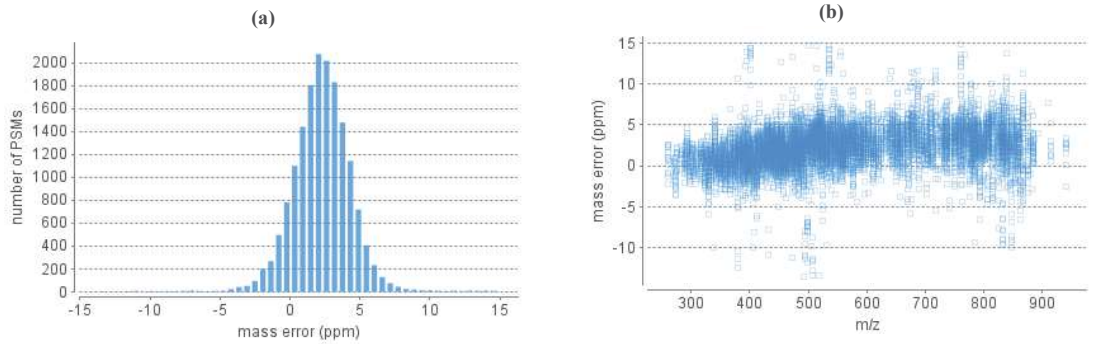

Table 5. Number of identified peptides in each sample by the number of missed cleavages

Missed Cleavages	0	1	2	3	4+
4aCA	0	0	0	0	6
4aEK	0	0	0	0	21
4aQ	0	0	0	0	8
4aRK	0	0	0	0	28
4a	0	0	0	0	33
4bCA	0	0	0	0	8
4bEK	0	0	0	0	15
4bQ	0	0	0	0	7
4bRK	0	0	0	0	3
4b	0	0	0	0	16
...					

4. Other Information

Table 6. Search parameters.

Search Engine Name: PEAKS
Parent Mass Error Tolerance: 15.0 ppm
Fragment Mass Error Tolerance: 0.01 Da
Precursor Mass Search Type: monoisotopic
Enzyme: None
Max Missed Cleavages: 100
Non-specific Cleavage: both
Max Variable PTM Per Peptide: 3
Database: TDP_237library_07202017
Taxon: All
Searched Entry: 228
FDR Estimation: Enabled
Different data refine parameters are used for this search:

Table 7. Instrument parameters.

Fractions: 4aCA_MY_pH46_60.raw, 4aEK_MY_pH46_60.raw, 4aQ_MY_pH46_60_200502050824.raw, 4aRK_MY_pH46_60_200501191944.raw, 4a_MY_pH46_60.raw, 4bCA_MY_pH46_60_200501172201.raw, 4bEK_MY_pH46_60_200501110132.raw, 4bQ_MY_pH46_60_200502011255.raw, 4bRK_MY_pH46_60_200501152411.raw, 4b_MY_pH46_60.raw, 4cCA_MY_pH46_60.raw, 4cEK_MY_pH46_60.raw, 4cQ_MY_pH46_60.raw, 4cRK_MY_pH46_60.raw, 4c_MY_pH46_60.raw, 4dCA_MY_pH46_60.raw, 4dEK_MY_pH46_60.raw, 4dQ_MY_pH46_60.raw, 4dRK_MY_pH46_60.raw, 4d_MY_pH46_60.raw, 7aCA_MY_pH72_60_200501211728.raw, 7aEK_MY_pH72_60.raw, 7aQ_MY_pH46_60_200502031039.raw, 7aRK_MY_pH72_60.raw, 7a_MY_pH72_60_200501231512.raw, 7bCA_MY_pH72_60_200502070608.raw, 7bEK_MY_pH72_60.raw, 7bQ_MY_pH46_60.raw, 7bRK_MY_pH72_60.raw, 7b_MY_pH72_60.raw, 7cCA_MY_pH72_60.raw, 7cEK_MY_pH72_60.raw, 7cQ_MY_pH46_60.raw, 7cRK_MY_pH72_60.raw, 7c_MY_pH72_60.raw, 7dCA_MY_pH72_60.raw, 7dEK_MY_pH72_60.raw, 7dQ_MY_pH46_60.raw, 7dRK_MY_pH72_60.raw, 7d_MY_pH72_60.raw, CAOnly.raw, EKOnly.raw, RKOnly.raw
Ion Source: ESI(nano-spray)
Fragmentation Mode: high energy CID (y and b ions)
MS Scan Mode: FT-ICR/Orbitrap
MS/MS Scan Mode: FT-ICR/Orbitrap