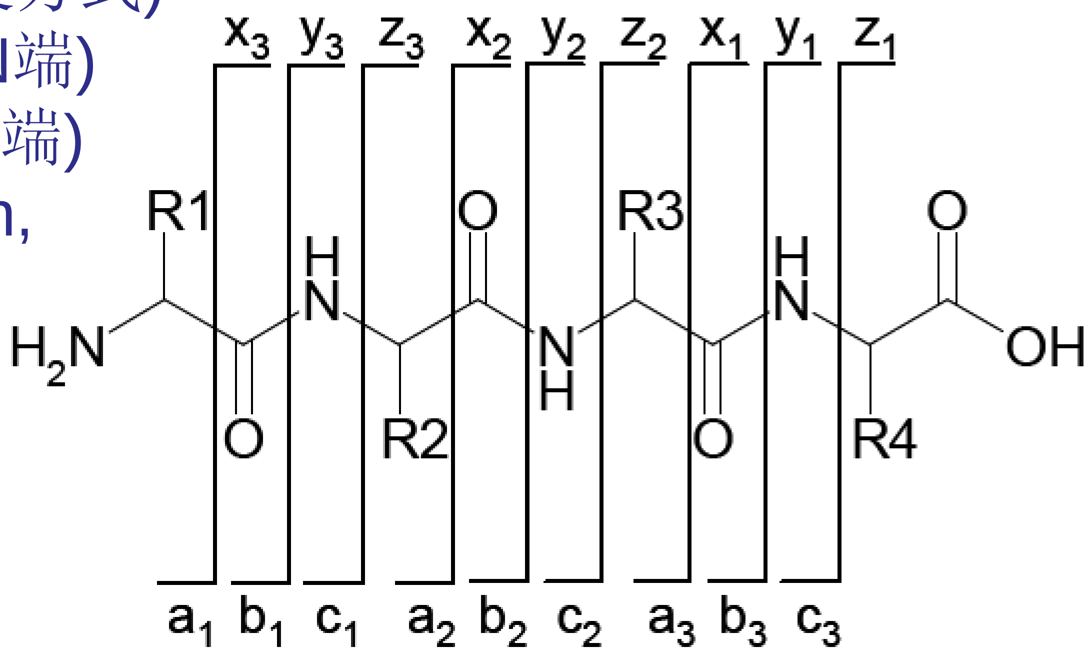
MALDI-TOF/TOF

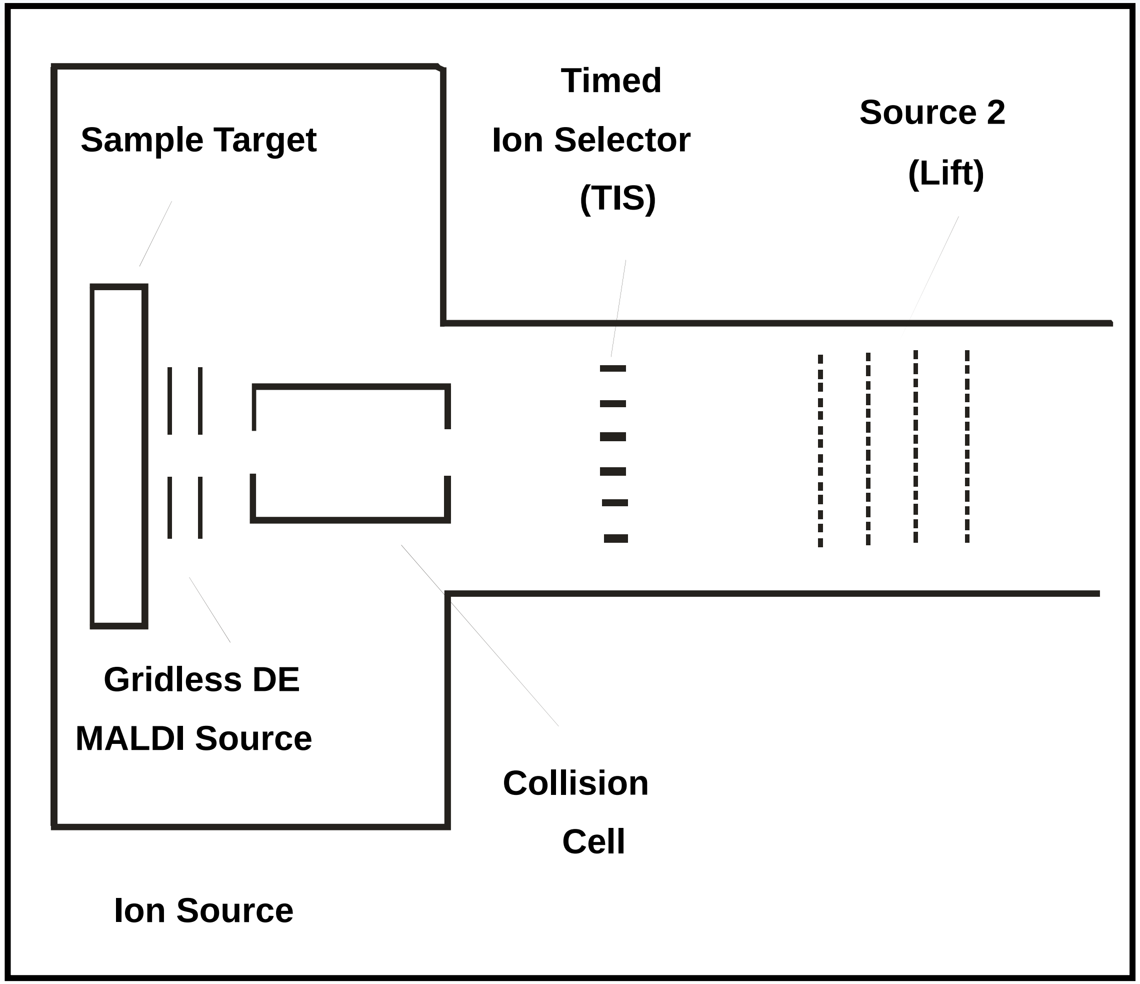
MS/MS中常见的碎片离子

- 主链断裂 (主要断裂方式)
  - a, b, c (电荷在N端)
  - x, y, z (电荷在C端)
  - I (immonium ion, NH2=CHR)



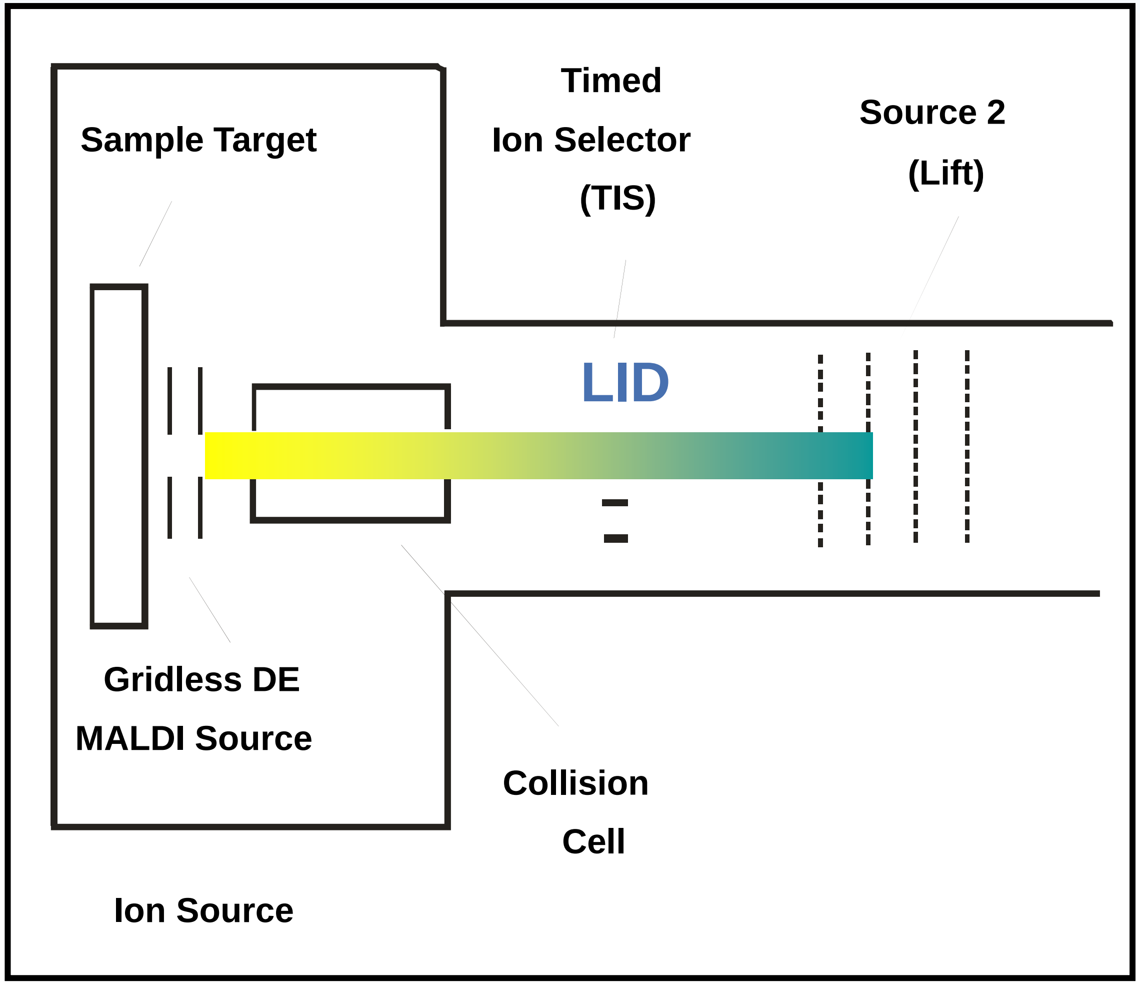
- 侧链断裂 (不常见)
  - d, w, v

解离方式



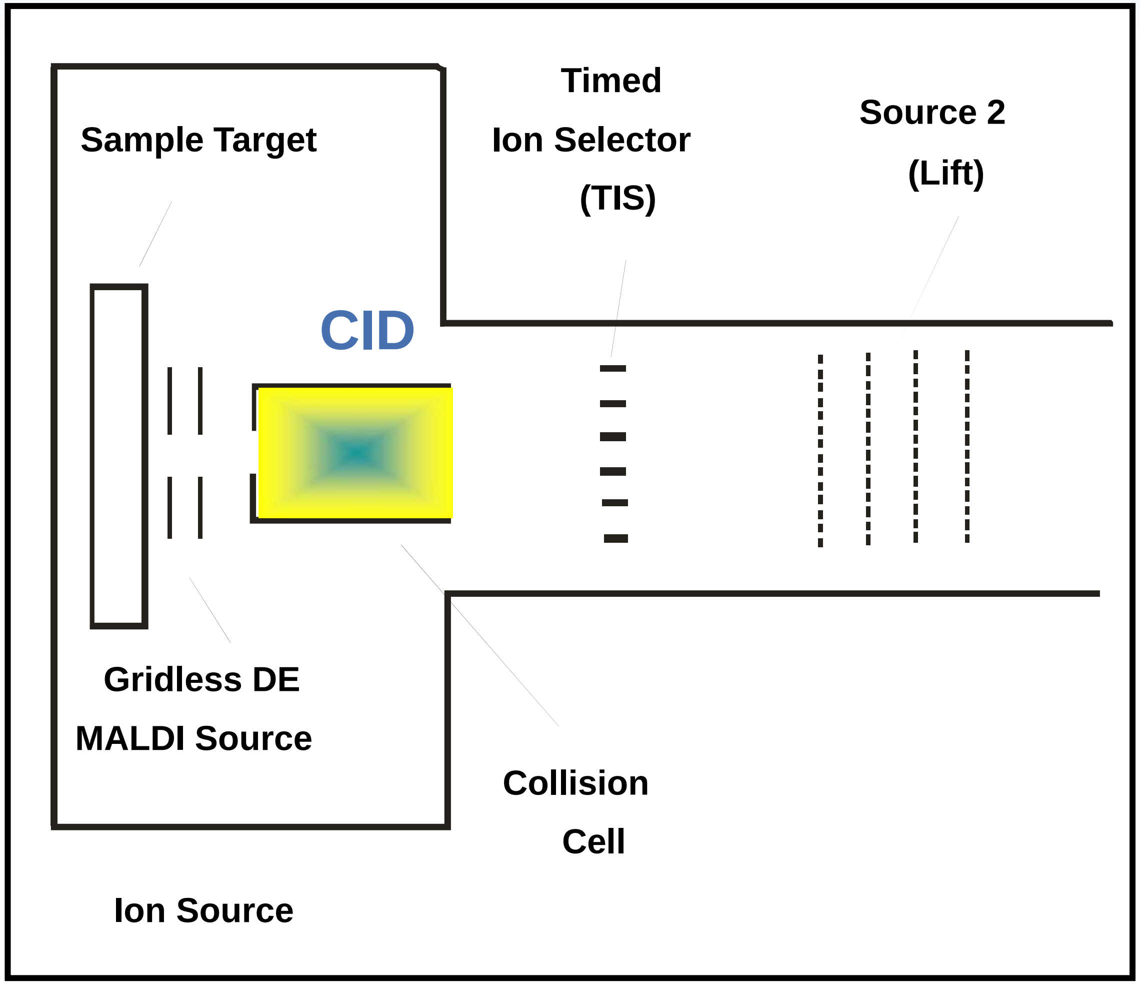
Sample Target
Timed
Ion Selector
(TIS)
Source 2
(Lift)
Gridless DE
MALDI Source
Collision
Cell
Ion Source

解离方式



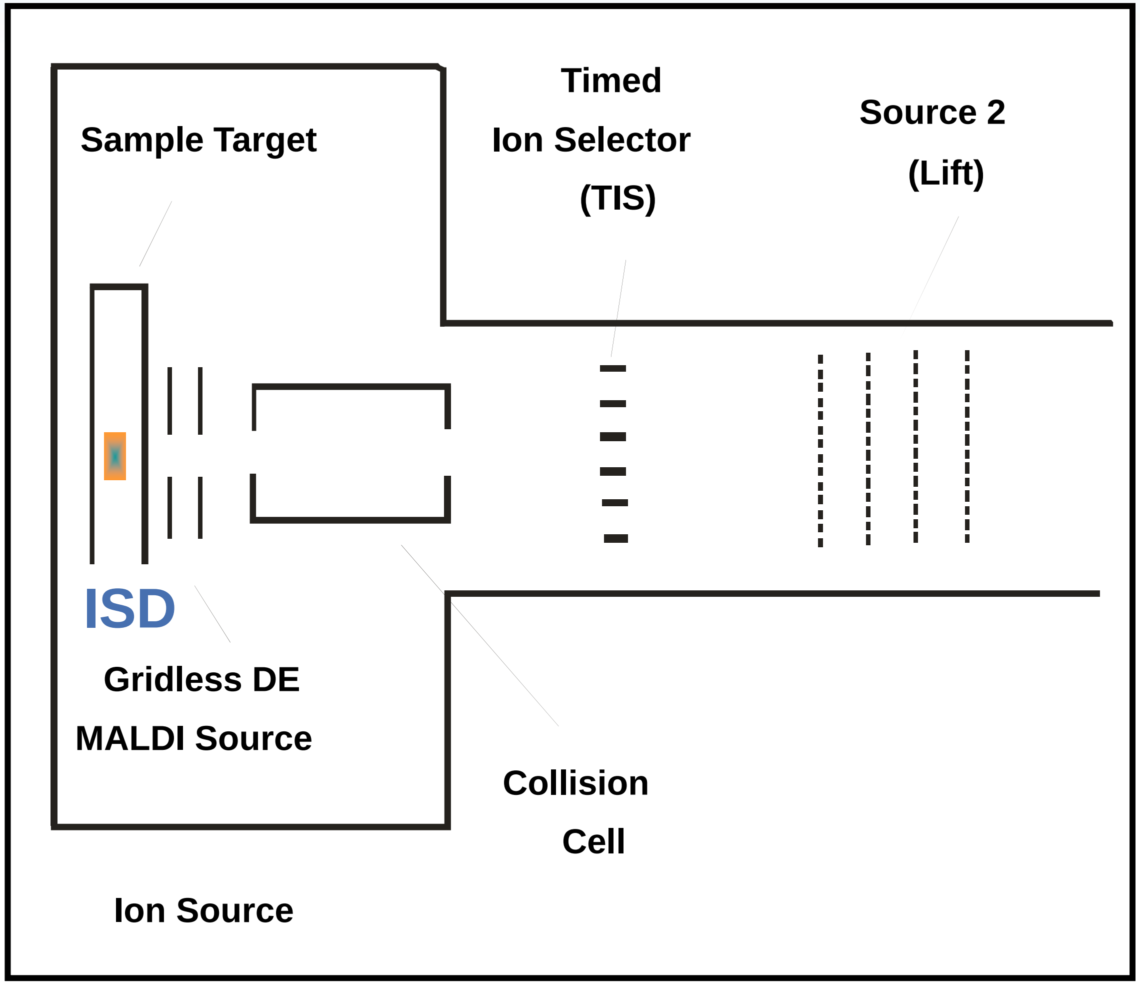
LID: Laser Induced Dissociation
(激光诱导解离)

解离方式



CID: Collision Induced Dissociation
(碰撞诱导解离)

解离方式



ISD: In Source Dissocation
(源内裂解)

LID vs. CID

BRUKER

LID: 激光诱导解离

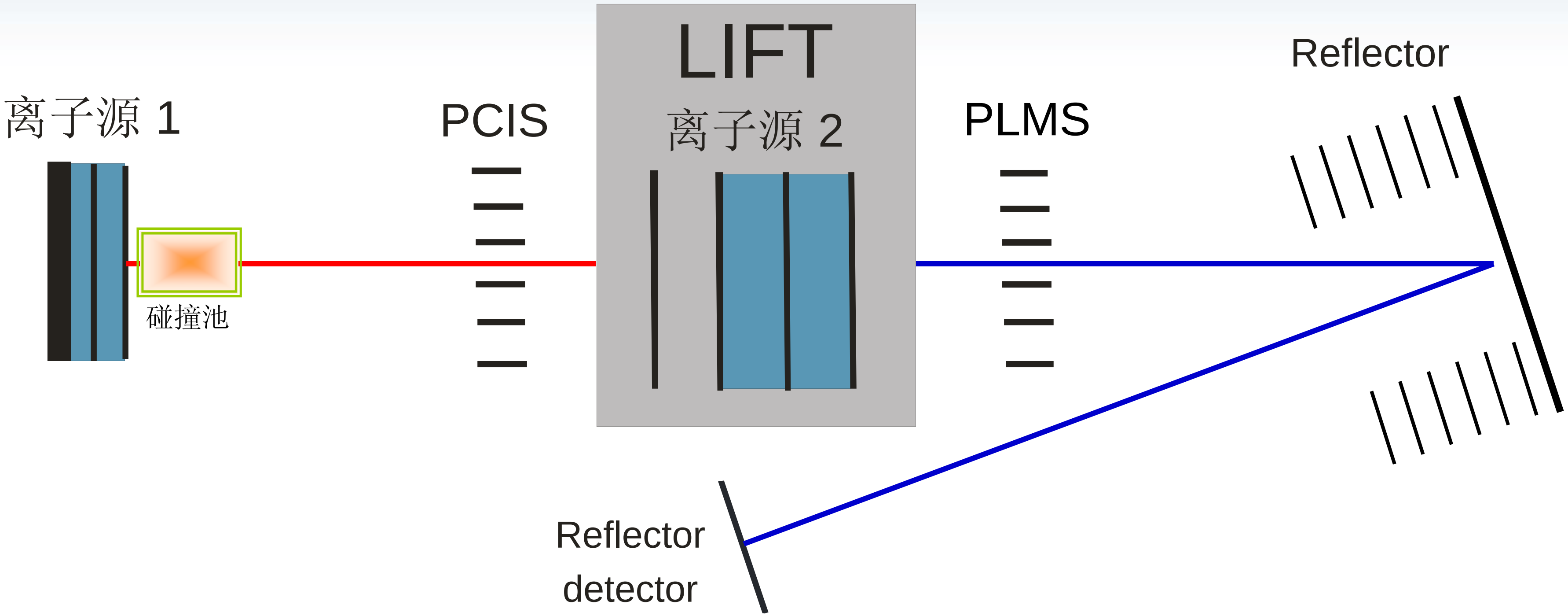
- 最直截了当的多肽主链解离模式(肽键的断裂)，通常形成b和y离子
- 常用于蛋白质鉴定

CID：碰撞诱导解离 (高能)

除了多肽主链解离模式外，也能观察到多肽支链断裂产物。内部碎片离子的信号强度更强。更倾向于生产低质量端的碎片离子。常用于：

- ***denovo sequencing*** **(**亚胺离子信号更强**)**
- 区分 ***L and I***
- *多糖分析* **(**确定多糖单体连接位点**)**

MALDI-TOF/TOF基本原理



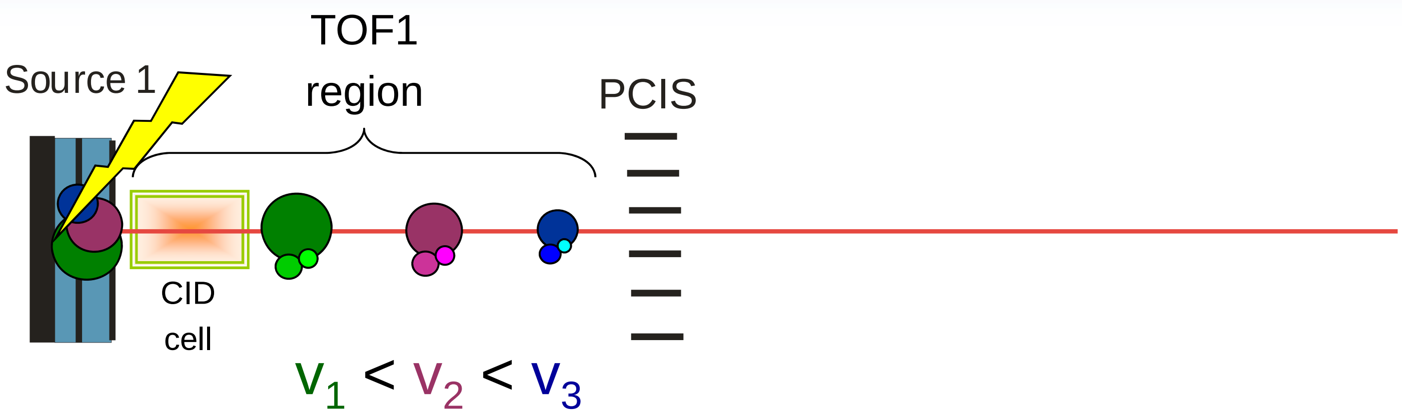
红色：离子在**TOF1(线性TOF)**的轨迹
蓝色：离子在**TOF2 (反射TOF)**的轨迹

离子源 **1 = MALDI ion source**
离子源 **2 = LIFT re-acceleration cell**

PCIS = Timed ion gate 母离子选择器
PLMS = Post LIFT metastable suppressor

MALDI-TOF/TOF

分析包含三种不同质合比的混合物 (green,red, blue)



通过第一级TOF，实现母离子分离

部分母离子在TOF1中裂解，裂解原因:

- 激光诱导亚稳态解离, metastable laser induced decay (**LID**)
- 碰撞诱导解离collision induced decay (**CID**)

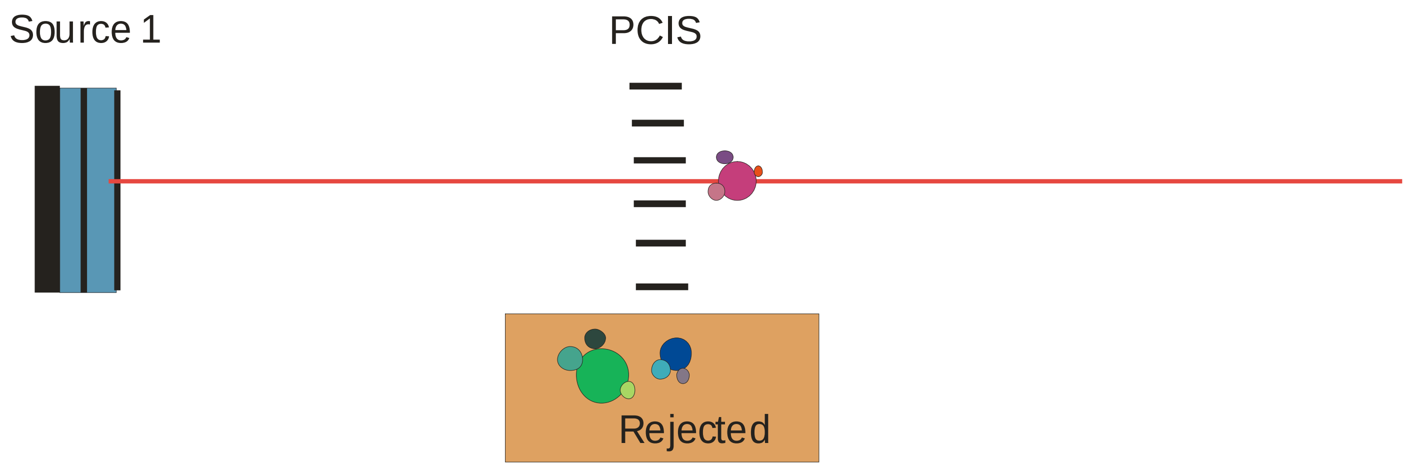
最重要的是:

碎片离子具有和其母离子一样的速度

MALDI-TOF/TOF

分析包含三种不同质合比的混合物 (**green**,**red**, **blue**)

选择红色**red**母离子做MS/MS碎片分析



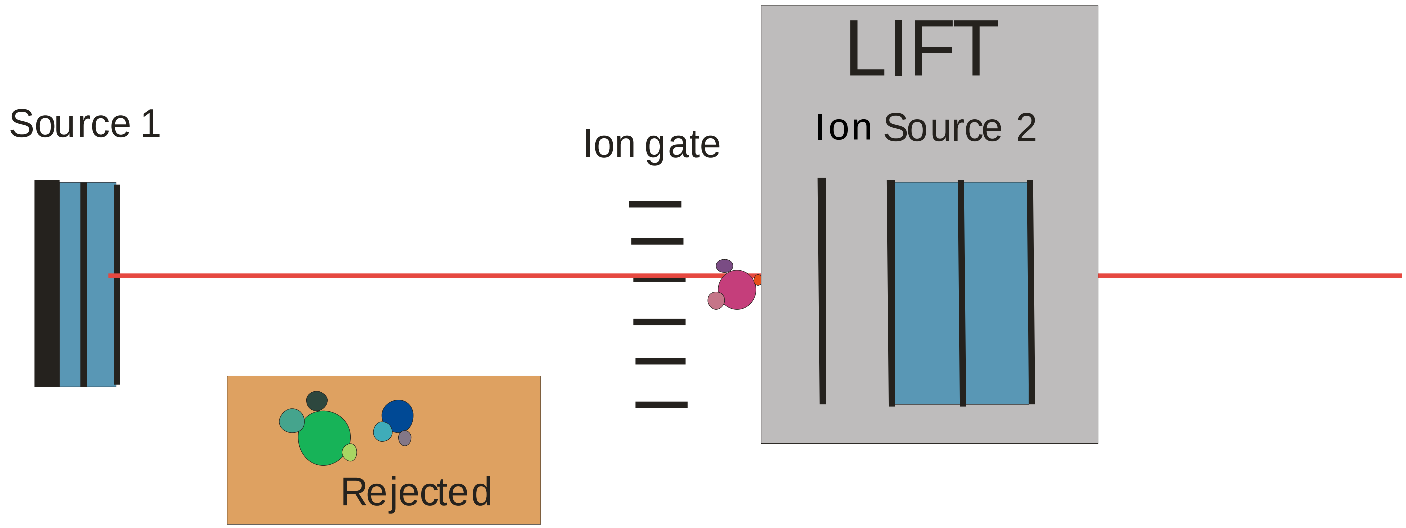
PCIS = Timed Ion Gate 母离子选择器

(calibrated for time/mass correlation)

MALDI-TOF/TOF

分析包含三种不同质合比的混合物 (green,red, blue)

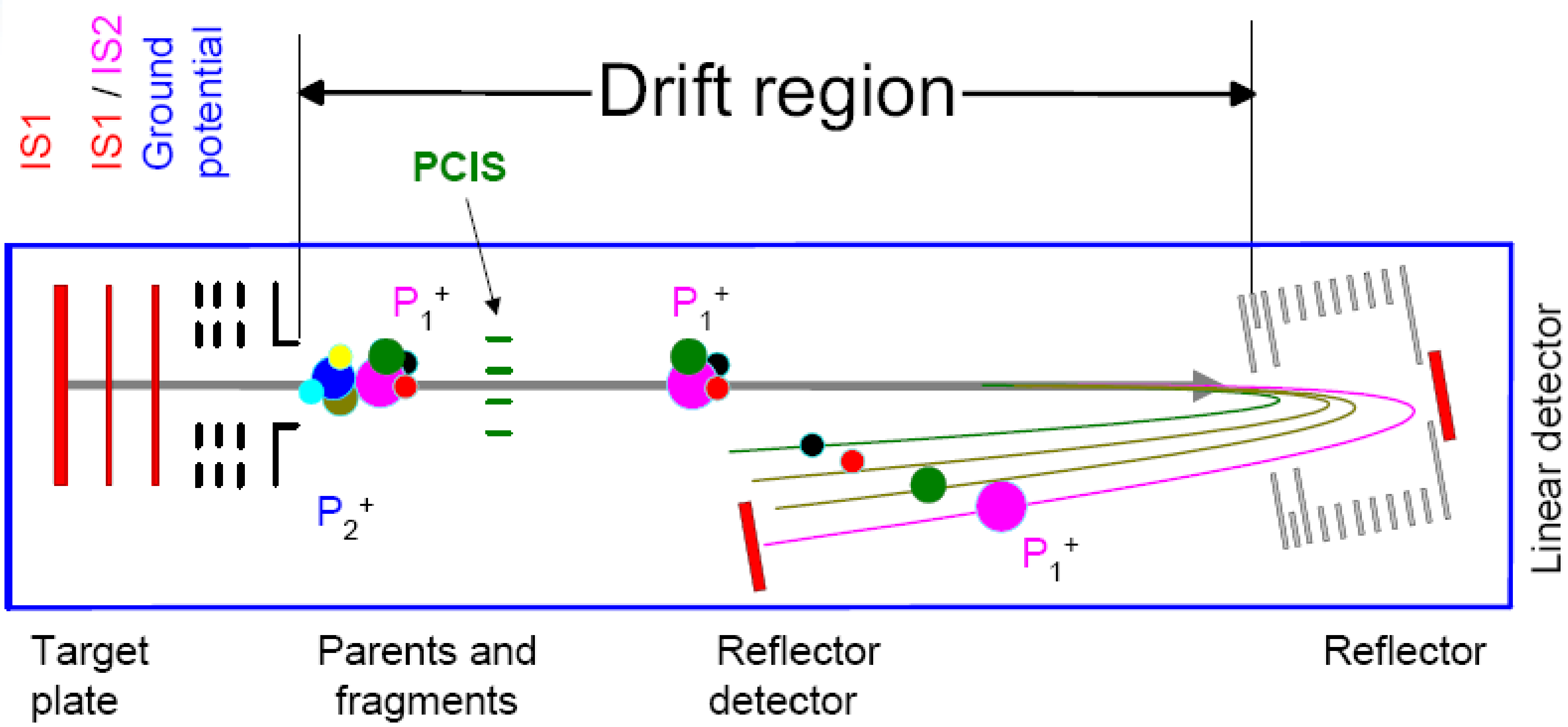
选择红色red母离子做MS/MS碎片分析



离子源2 (LIFT):

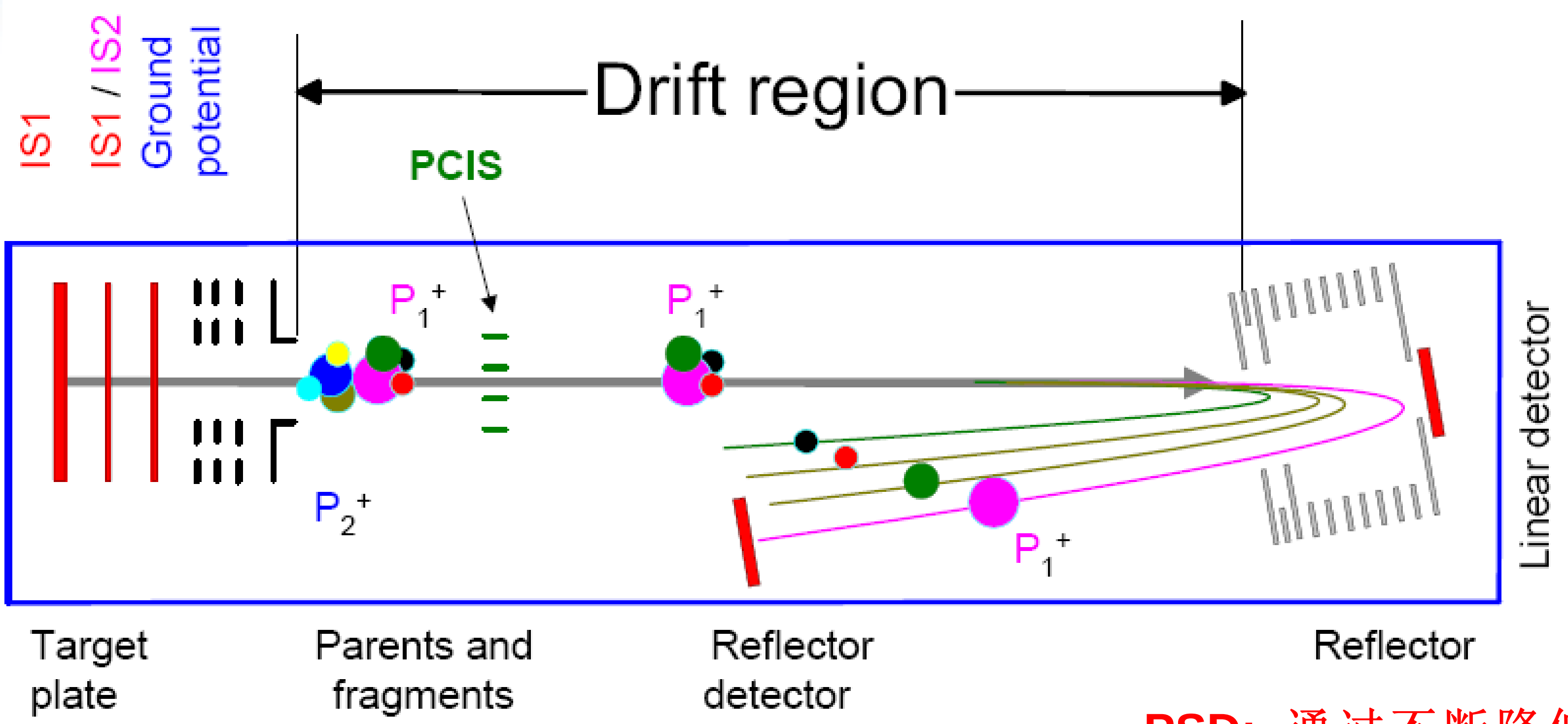
- 对母离子及其子离子进行再加上(二级加速),从而区分不同分子量的子离子
- 对子离子再聚焦

MALDI-TOF/TOF基本原理



PCIS = Timed ion gate 母离子选择器

MALDI-TOF/TOF基本原理

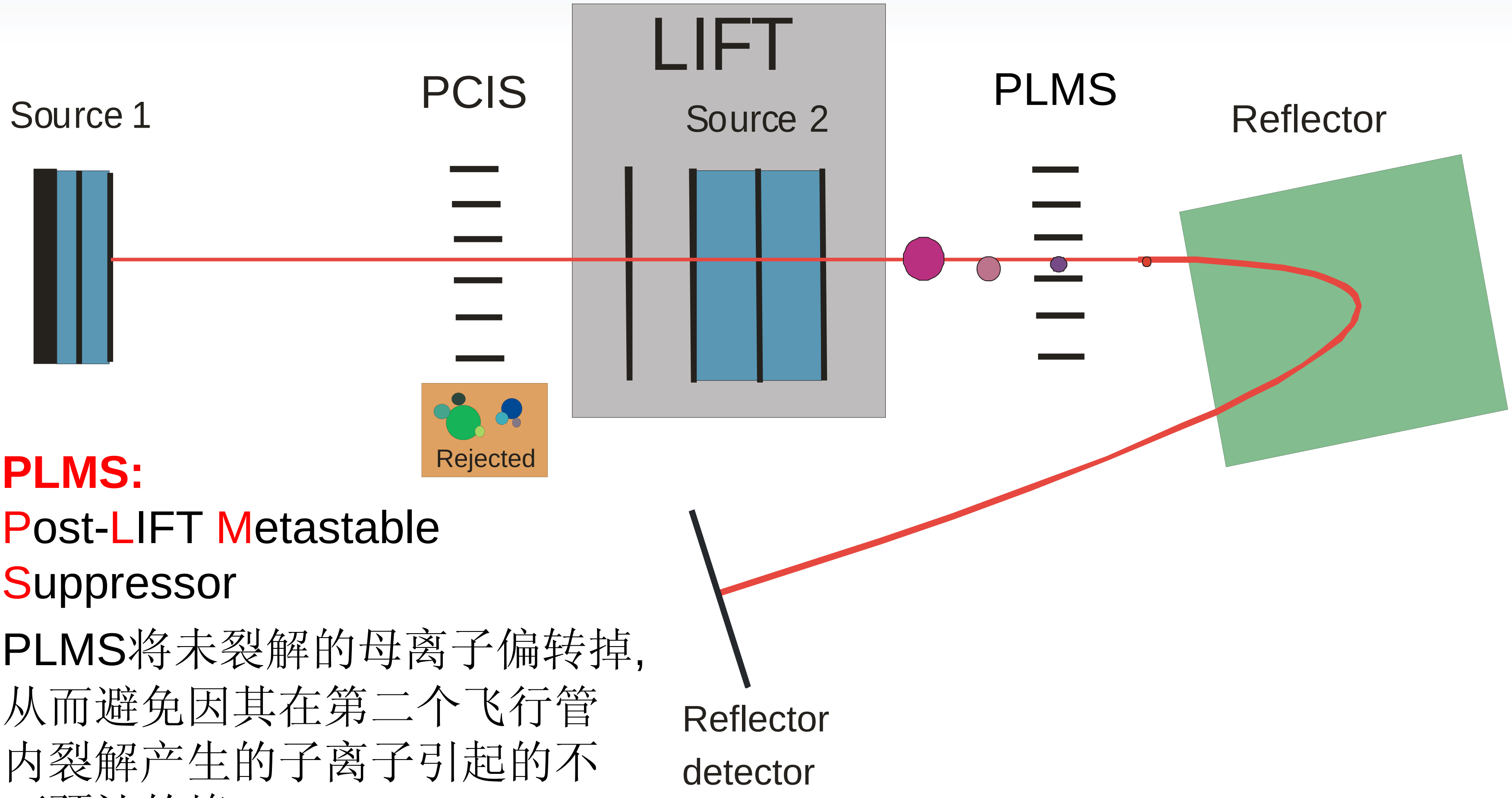


PSD：通过不断降低反射器电压，将不同分子量的碎片离子以此聚焦到检测器

PCIS = Timed ion gate 母离子选择器

MALDI-TOF/TOF

分析包含三种不同质合比的混合物 (green,red, blue)



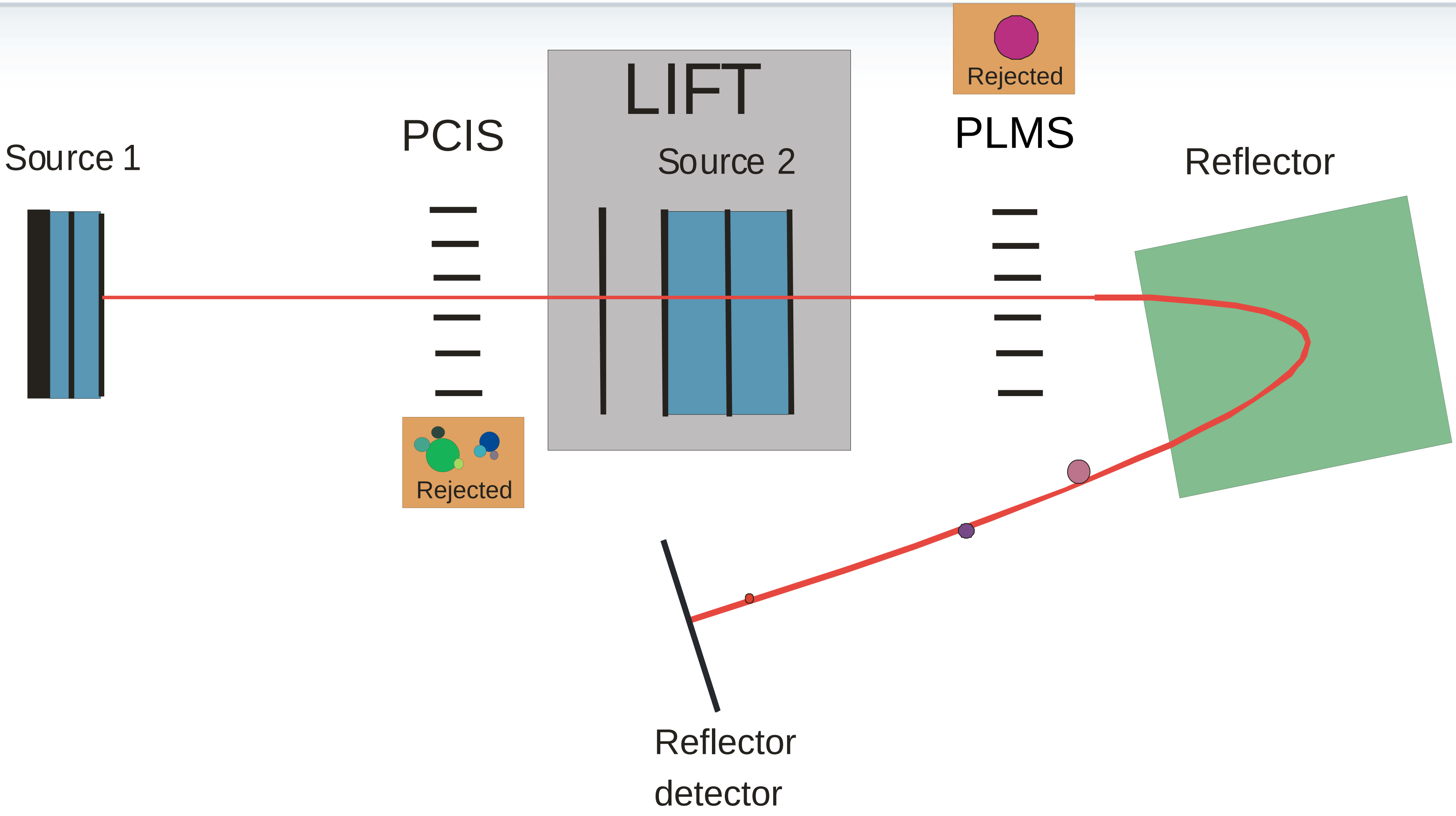
PLMS:

Post-LIFT Metastable Suppressor

PLMS将未裂解的母离子偏转掉，从而避免因其在第二个飞行管内裂解产生的子离子引起的不可预计的峰

MALDI-TOF/TOF

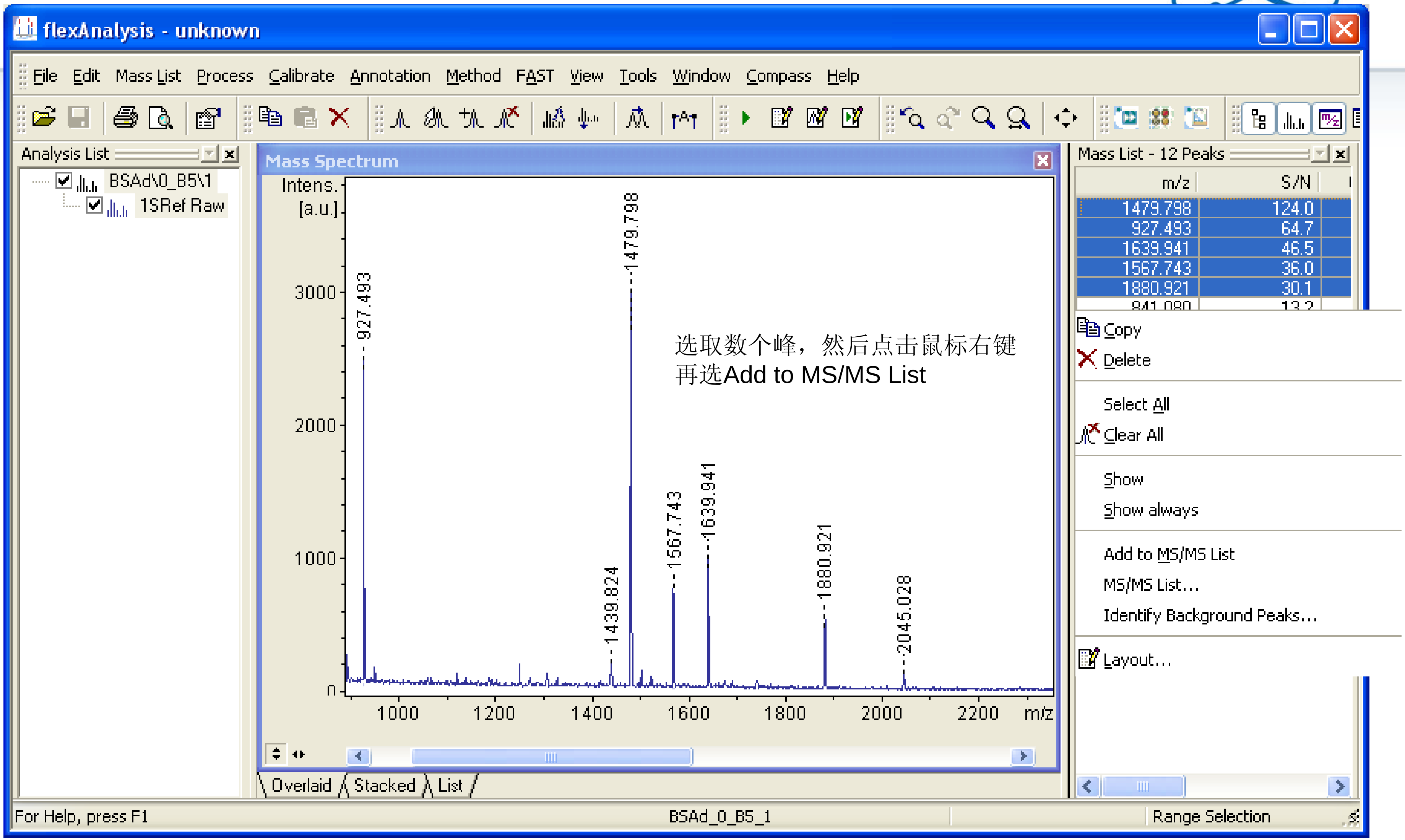
分析包含三种不同质合比的混合物 (green,red, blue)



TOF/TOF数据采集流程

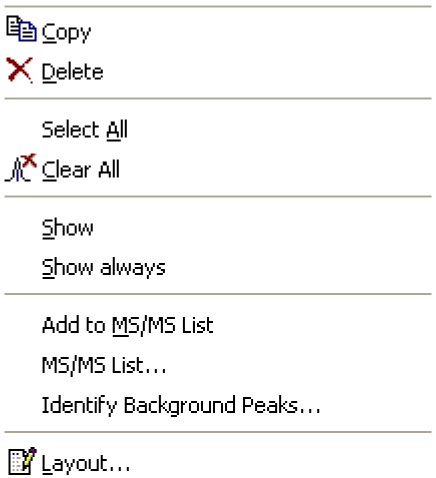
- 获取PMF图
- 从PMF图里选取一些信噪比比较高的峰做TOF/TOF
- 选择LIFT方法
- 获取母离子质谱(parent mode)
- 获取子离子质谱(fragment mode)
- 保存数据

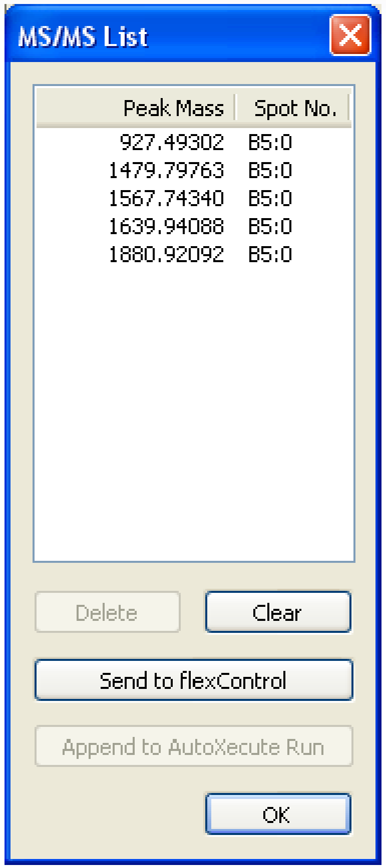
PMF图


flexAnalysis - unknown
File Edit Mass List Process Calibrate Annotation Method FAST View Tools Window Compass Help
Analysis List
BSAd\0_B5\1
1SRef Raw
Mass Spectrum
Intens. [a.u.]
3000
2000
1000
0
927.493
1479.798
1439.824
1567.743
1639.941
1880.921
2045.028
1000 1200 1400 1600 1800 2000 2200 m/z
选取数个峰，然后点击鼠标右键
再选Add to MS/MS List
Overlaid Stacked List
Mass List - 12 Peaks
m/z S/N
1479.798 124.0
927.493 64.7
1639.941 46.5
1567.743 36.0
1880.921 30.1
Copy
Delete
Select All
Clear All
Show
Show always
Add to MS/MS List
MS/MS List...
Identify Background Peaks...
Layout...
For Help, press F1
BSAd_0_B5_1
Range Selection

选取母离子

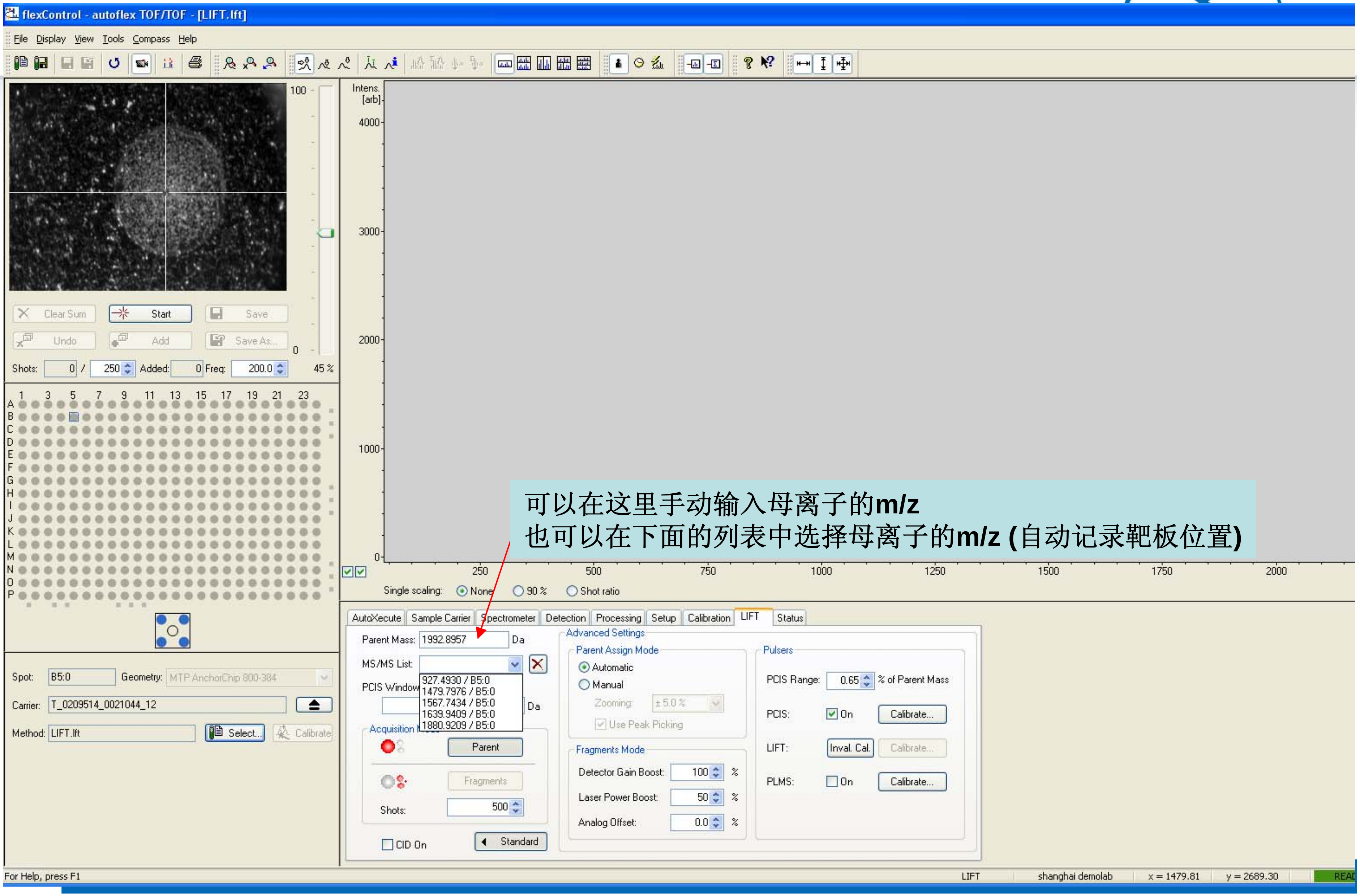
再点击鼠标右键
选取MS/MS List….
弹出右边窗口



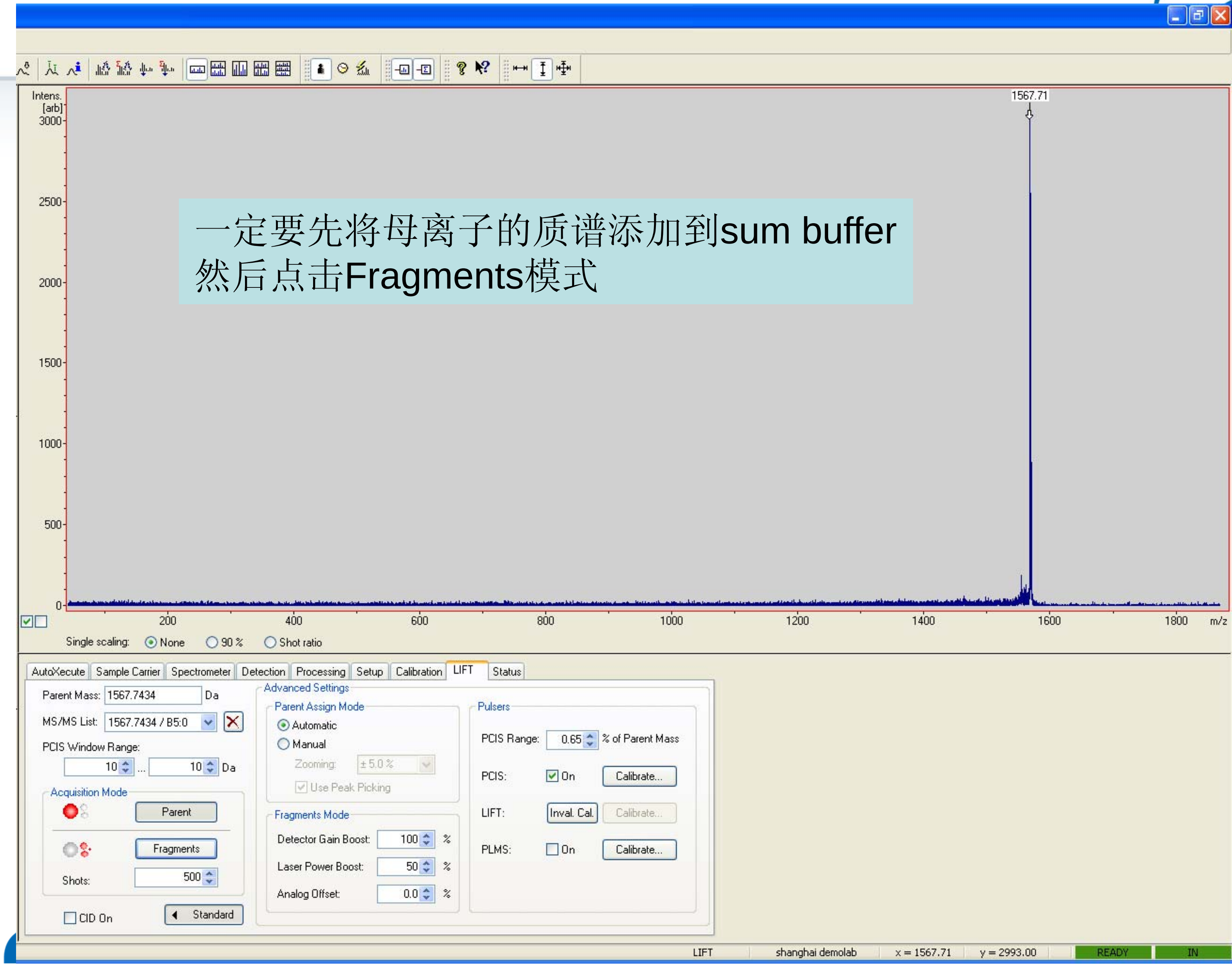


点击Send to flexControl

选择LIFT方法

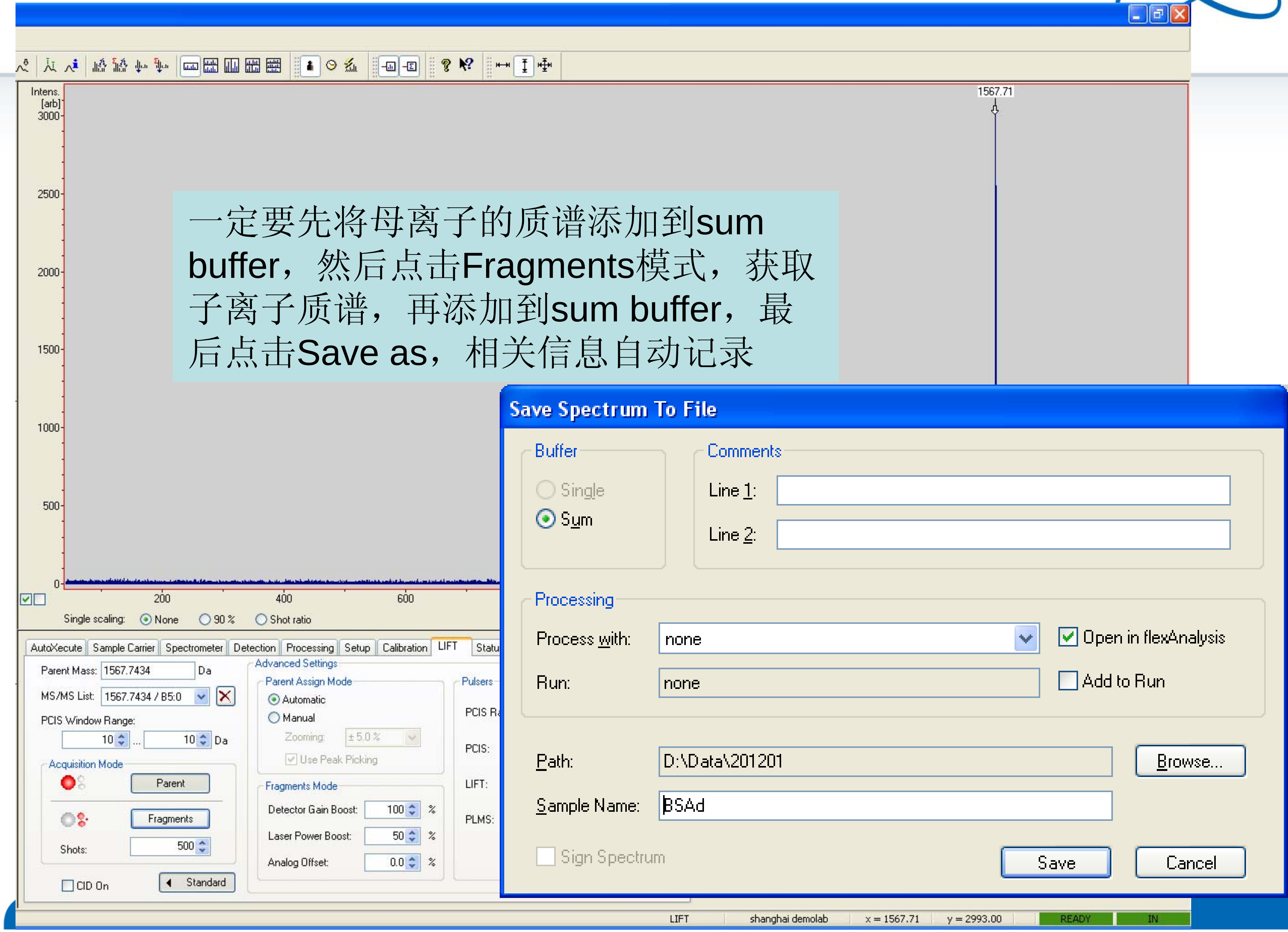

flexControl - autoflex TOF/TOF - [LIFT.lft]
File Display View Tools Compass Help
Clear Sum
Start
Save
Undo
Add
Save As...
Shots: 0 / 250 Added: 0 Freq: 200.0 45 %
Intens. [arb]
Single scaling: None 90 % Shot ratio
AutoXecute Sample Carrier Spectrometer Detection Processing Setup Calibration LIFT Status
Parent Mass: 1992.8957 Da
MS/MS List
927.4930 / B5:0
1479.7976 / B5:0
1567.7434 / B5:0
1639.9409 / B5:0
1880.9209 / B5:0
PCIS Window
Parent
Fragments
Shots: 500
CID On
Standard
Advanced Settings
Parent Assign Mode
Automatic
Manual
Zooming: ± 5.0 %
Use Peak Picking
Fragments Mode
Detector Gain Boost: 100 %
Laser Power Boost: 50 %
Analog Offset: 0.0 %
Pulsers
PCIS Range: 0.65 % of Parent Mass
PCIS: On Calibrate...
LIFT: Inval. Cal. Calibrate...
PLMS: On Calibrate...
Spot: B5:0
Geometry: MTP AnchorChip 800-384
Carrier: T_0209514_0021044_12
Method: LIFT.lft
Select...
Calibrate
For Help, press F1
LIFT
shanghai demolab
x = 1479.81
y = 2689.30
可以在这里手动输入母离子的m/z
也可以在下面的列表中选择母离子的m/z (自动记录靶板位置)

获取母离子的m/z


一定要先将母离子的质谱添加到sum buffer
然后点击Fragments模式
1567.71
Intens. [arb]
m/z
Single scaling: None 90 % Shot ratio
AutoXecute Sample Carrier Spectrometer Detection Processing Setup Calibration LIFT Status
Parent Mass: 1567.7434 Da
MS/MS List: 1567.7434 / B5:0
PCIS Window Range:
Acquisition Mode
Parent
Fragments
Shots: 500
CID On
Standard
Advanced Settings
Parent Assign Mode
Automatic
Manual
Zooming
Use Peak Picking
Fragments Mode
Detector Gain Boost: 100 %
Laser Power Boost: 50 %
Analog Offset: 0.0 %
Pulsers
PCIS Range: 0.65 % of Parent Mass
PCIS: On Calibrate...
LIFT: Inval. Cal. Calibrate...
PLMS: On Calibrate...
LIFT shanghai demolab x = 1567.71 y = 2993.00 READY IN

获取母离子的m/z

一定要先将母离子的质谱添加到sum buffer，然后点击Fragments模式，获取子离子质谱，再添加到sum buffer，最后点击Save as，相关信息自动记录



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