

Skyline 使用

—— 筛选合适的肽段和离子

生物制药小组

Review the latest Skyline Tutorial Webinar

Ion Mobility Spectrum Filtering in Skyline

Use audit logging in Skyline. Learn how to produce fully audit logged Skyline documents to help others reproduce your research, and you to remember what you did, helping with the writing of your methods sections for publication. Learn how to work with the Audit Log grid view, an extension of the Document Grid to explore logged changes and provide reasons for the changes you make. This example uses a calibration experiment to show logging of settings changes, data import, integration adjustments, and exclusion of points from a calibration curve. Finally, you will upload your document to Panorama and see how the audit log is captured for review through a browsable web interface. (23 pages)

Download & Install:

Skyline 21.1 - 64 bit

Skyline-daily (beta)

VIDEO TRAILER ▶

External Tools



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1. Skyline 软件安装
2. Skyline 筛选特征肽段
3. Skyline 优化 CE

1. Skyline 软件安装

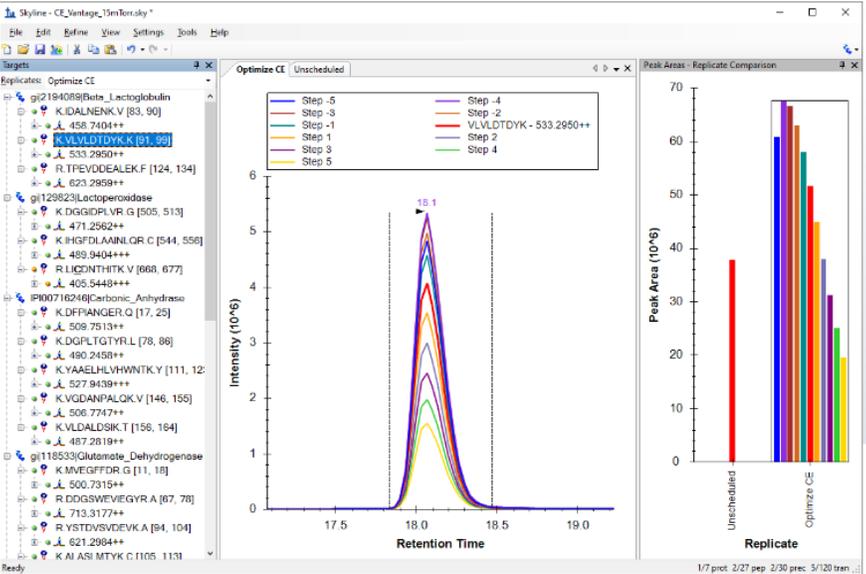
Skyline 软件安装

1. 在 Google 上搜索 Skyline peptide , 进入下载网页, 下载 skyline(有安装包), 安装。

<https://skyline.ms/project/home/software/Skyline/begin.view>

Watch presentations from the **Skyline User Group Meeting**
Online over two days - May 27 and 28th, 2020

Review the latest Skyline Tutorial Webinar
Ion Mobility Spectrum Filtering in Skyline



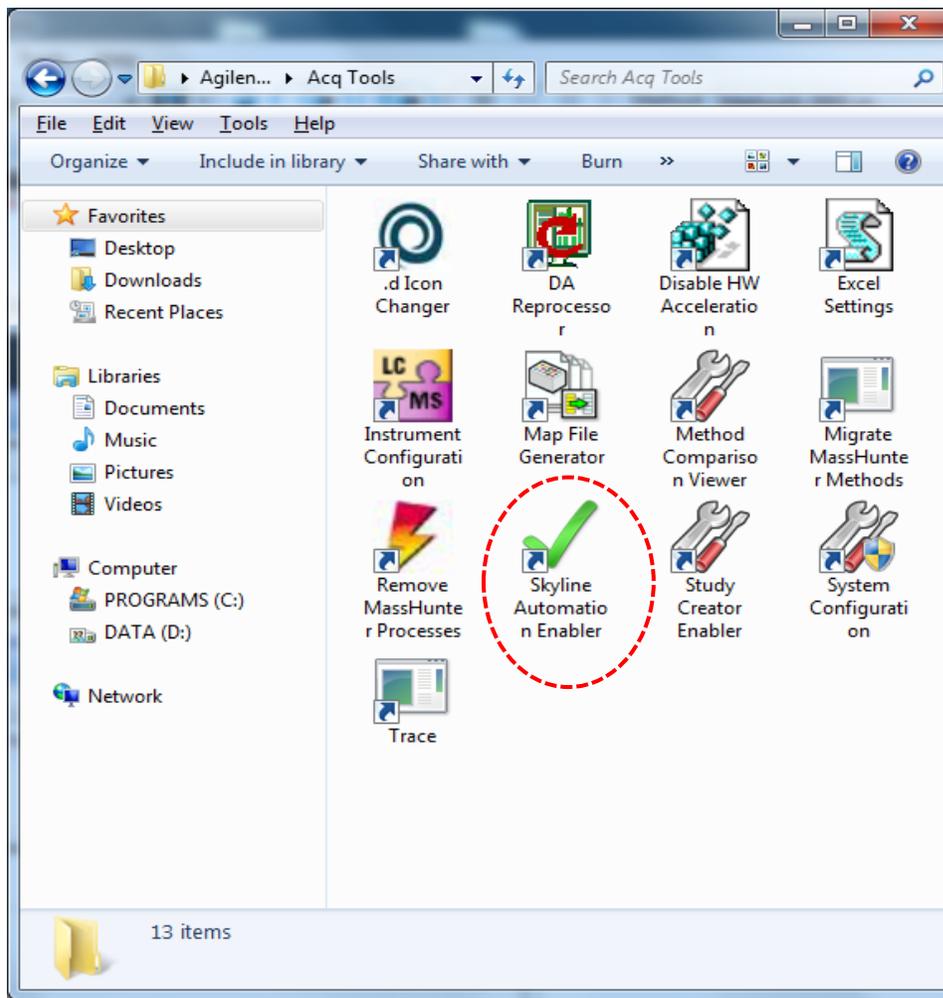
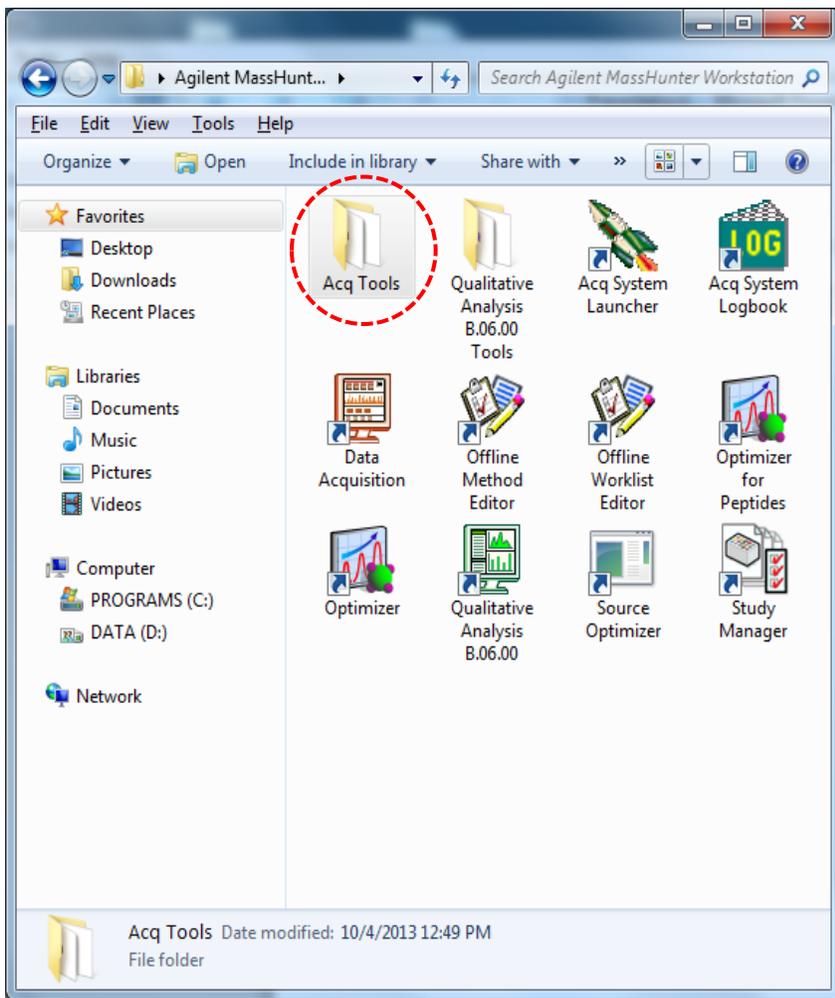
The screenshot shows the Skyline software interface. On the left, a list of peptide sequences is displayed, including K.IDALNENK.V [83, 90] and K.YAAELHLVHWNTK.V [111, 12]. The main window displays a chromatogram with Intensity (10⁶) on the y-axis and Retention Time on the x-axis. A peak is labeled at 18.1 minutes. To the right, a bar chart titled 'Peak Areas - Replicate Comparison' shows the peak area for 'Unscheduled' and 'Optimize CE' replicates. The 'Optimize CE' replicates show significantly higher peak areas compared to the 'Unscheduled' replicate.

Use Skyline to optimize collision energy (CE) values. Create scheduled CE optimization transitions lists for a document with 30 peptide precursors. Using supplied RAW files from a Thermo TSQ Vantage, you will recalculate the linear equation used to calculate CE for that instrument. You will also export a transition list with CE values optimized separately for each transition. (12 pages)

Download & Install:

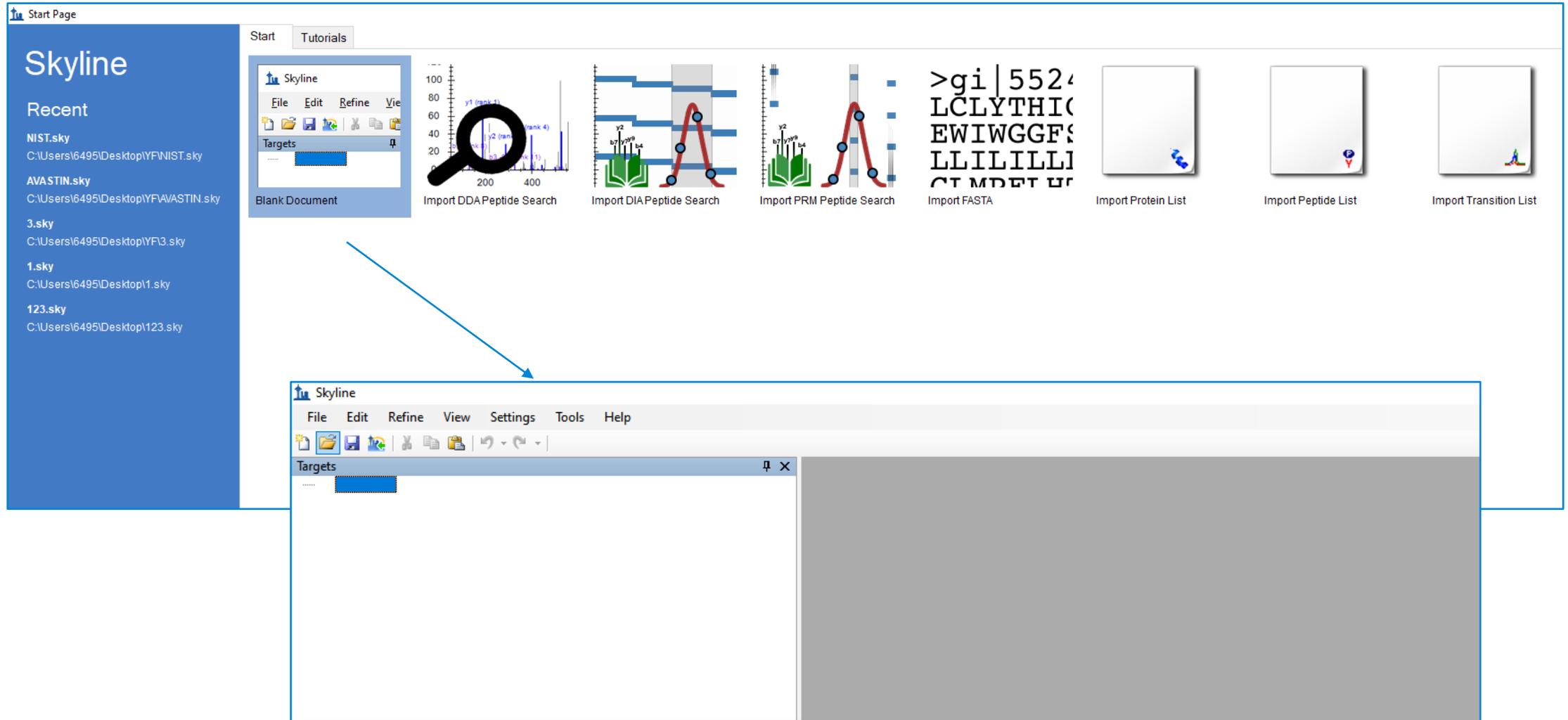
- Skyline 21.1 - 64 bit
- Skyline-daily (beta)
- VIDEO TRAILER ▶
- External Tools

2. 安装后在 Agilent MassHunter 中查看



2. Skyline 筛选特征肽段

1. 打开 Skyline 软件



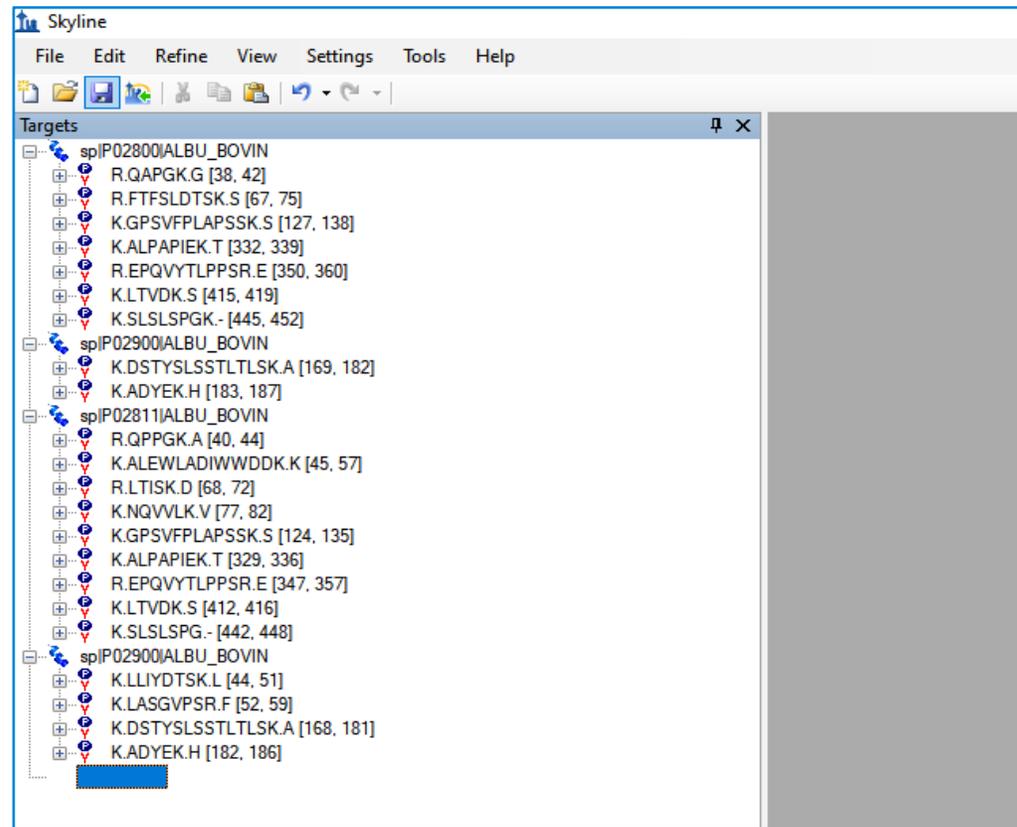
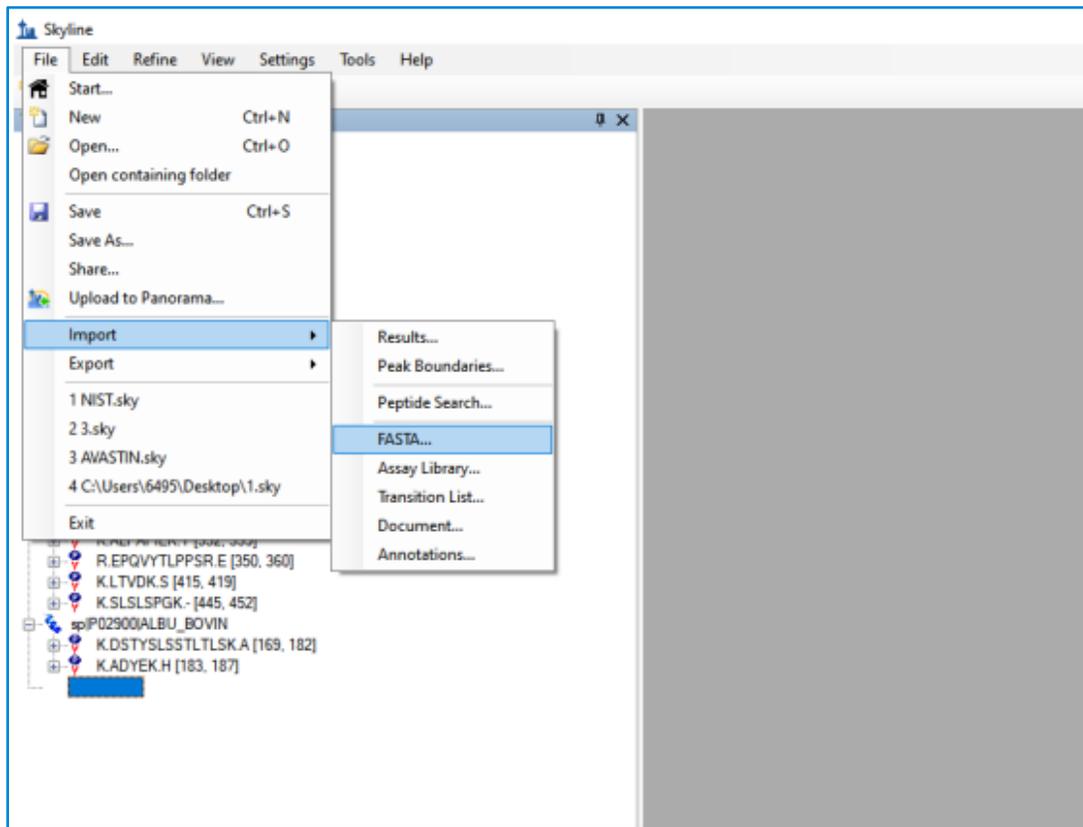
2. 筛选人血液中的特征肽段

```
BEVA.fasta - Notepad
File Edit Format View Help
>sp|P02800|ALBU_BOVIN Albumin OS=Bos taurus OX=9913 GN=ALB PE=1 SV=4
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FPAVQLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
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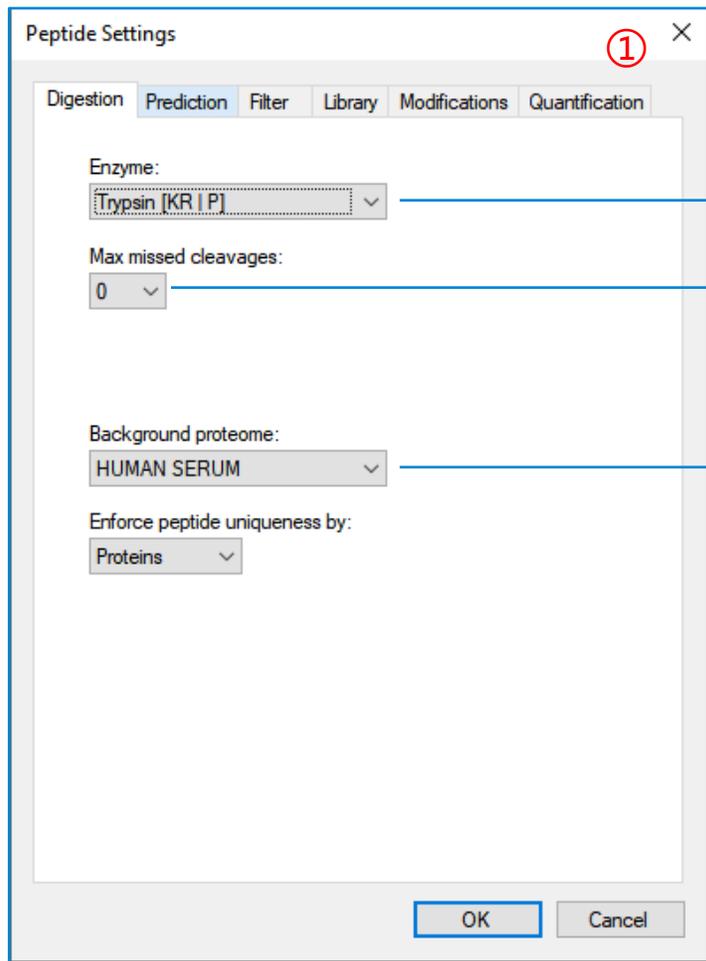
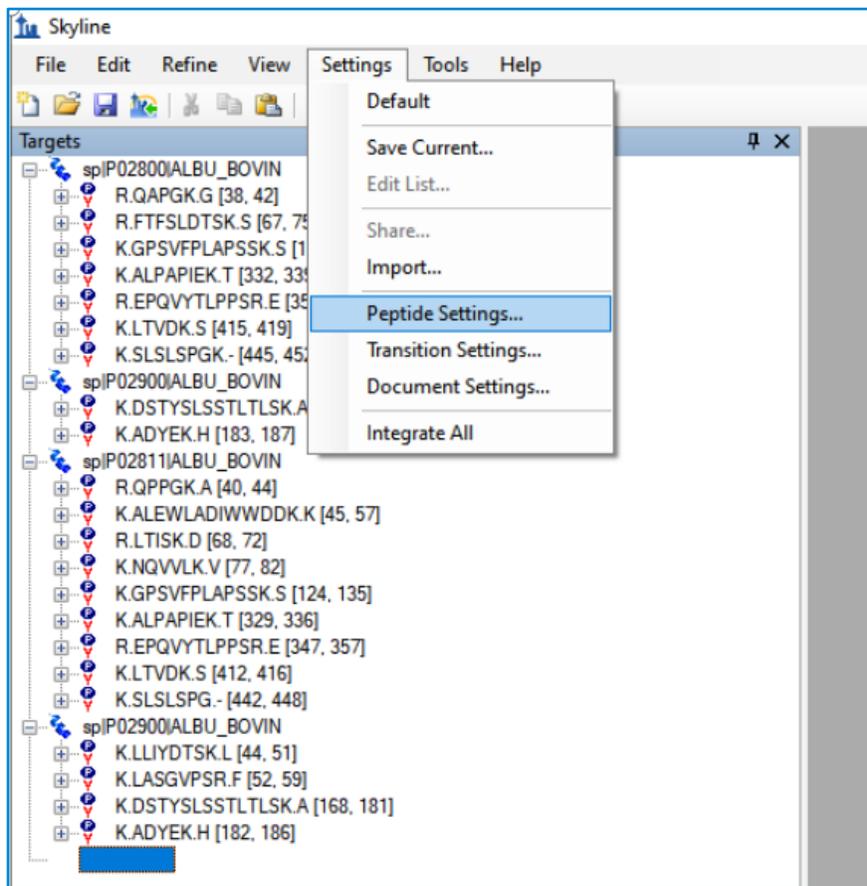
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SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

BEVA.fasta	5/11/2021 4:05 PM	Text Document	1 KB
NIST.fasta	5/12/2021 11:34 AM	Text Document	1 KB
uniprot-human+serum.fasta	5/11/2021 5:24 PM	FASTA File	6,312 KB

(1) 将需要定量的抗体氨基酸序列转化成如图 fasta格式，这个格式可以直接导入到 skyline 软件中；另外，需要在 <https://www.uniprot.org/> 网站下载人血清蛋白库，便于查找目的蛋白特征肽段。



(2) 打开Skyline, 导入目的蛋白序列。

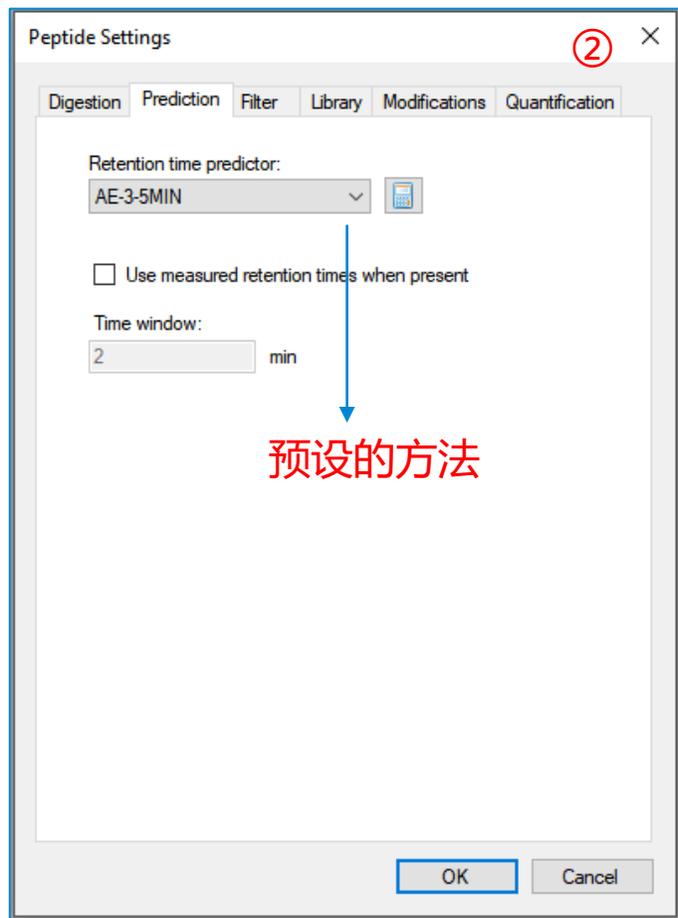


选择处理蛋白的酶

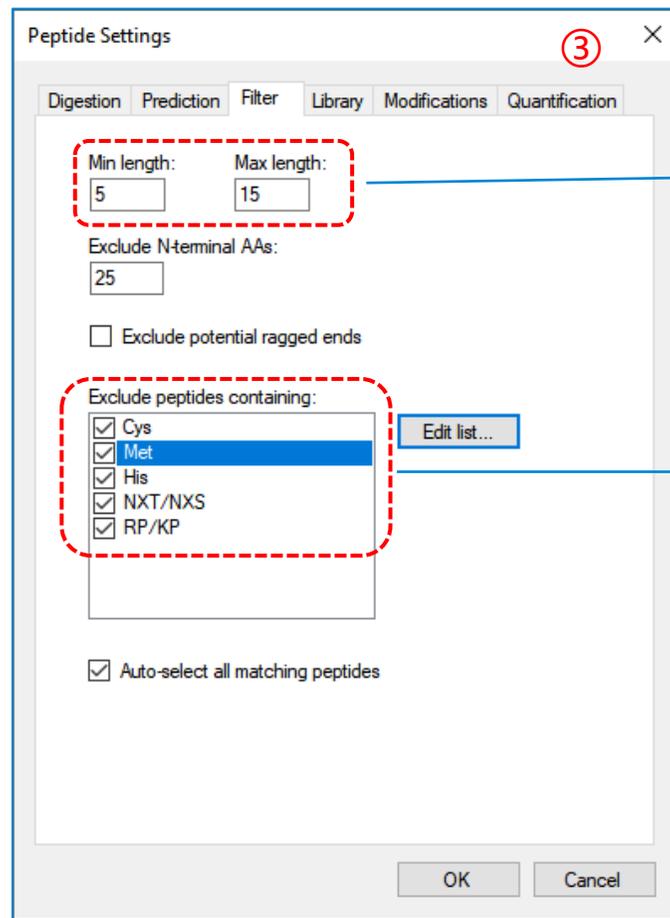
允许漏切位点的个数

基质中可能存在的蛋白组，在 [Uniprot](http://www.uniprot.org/) 网站中查找

(3) 打开Skyline，导入目的蛋白序列；在 Settings 中选择 Peptide Settings 设置参数。



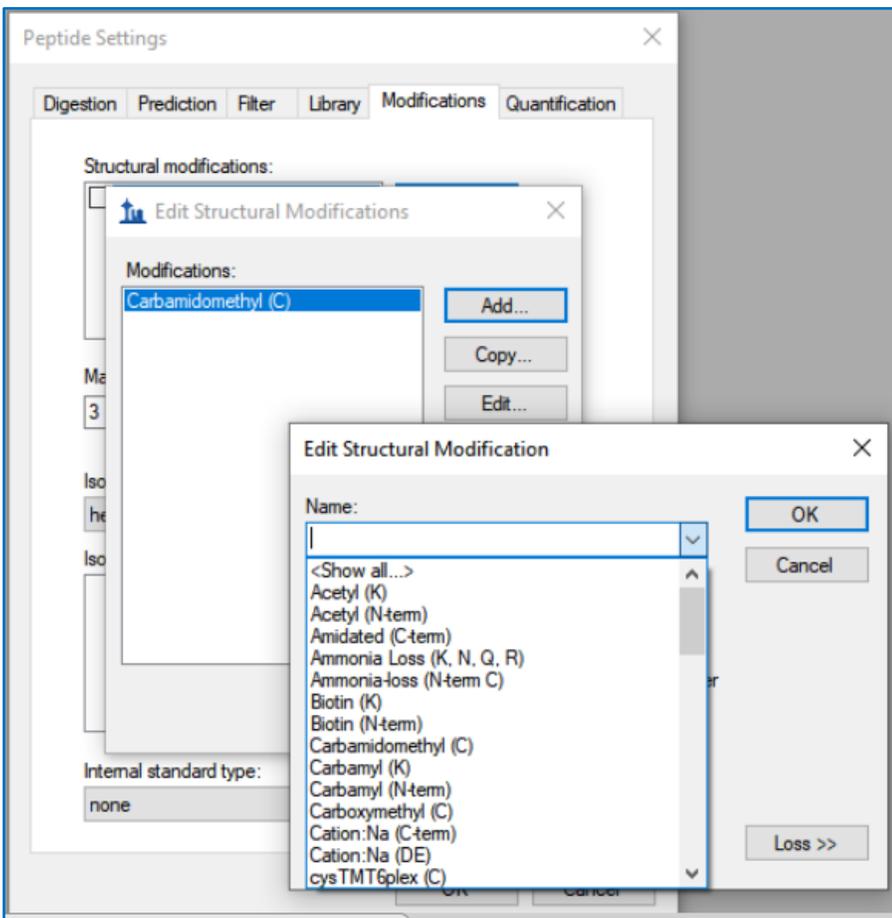
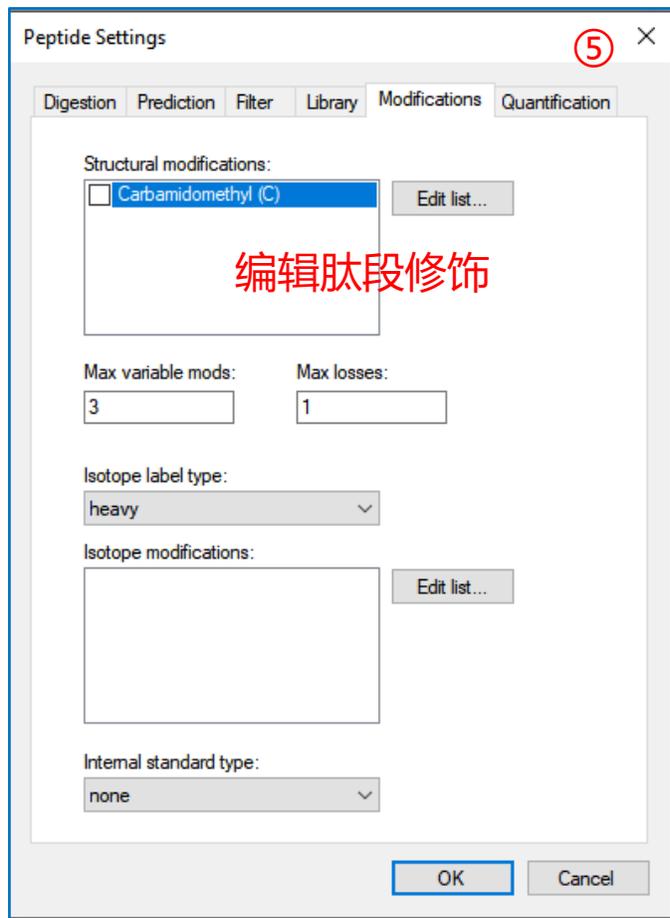
预设的方法



选择肽段最小和最大长度

选择不包括以下氨基酸的肽段

(4) 设置 Peptide Settings 参数。



可以选择修饰，但是这次我们需要的肽段最好不含修饰。

(5) 设置 Peptide Settings 参数。

Skyline - BAVA.sky
 File Edit Refine View Settings Tools Help
 Settings
 Default
 Save Current...
 Edit List...
 Share...
 Import...
 Peptide Settings...
Transition Settings...
 Document Settings...
 Integrate All

Transition Settings ①
 Prediction Filter Library Instrument Full-Scan Ion Mobility
 Precursor mass: Average
 Product ion mass: Average
 Collision energy: Agilent QQQ
 Declustering potential: None
 Optimization library: None
 Compensation voltage: None
 Use optimization values when present
 Optimize by: Transition
 OK Cancel

Collision energy: Agilent QQQ
 Declustering potential: None
 Compensation voltage: None
 Agilent QQQ
 None
 Agilent QQQ
 SCIEX
 Shimadzu QQQ
 Thermo TSQ Quantiva
 Thermo TSQ Ultra
 Thermo TSQ Vantage
 Waters Xevo
 <Add...>
 <Edit current...>
 <Edit list...>
 Transition

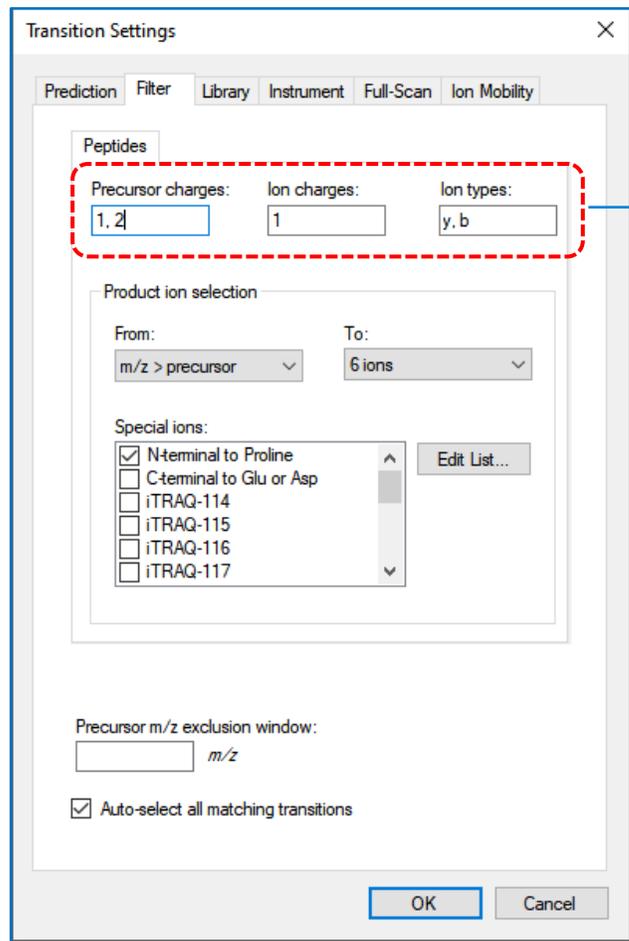
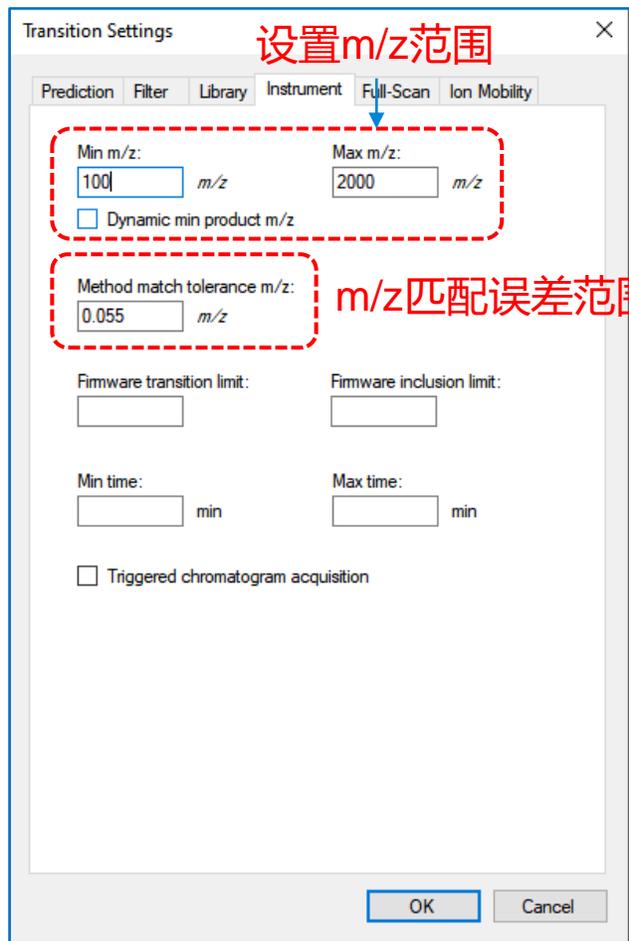
Edit Collision Energy Equation
 Name: Agilent QQQ
 OK
 Cancel
 Regression parameters:

	Charge	Slope	Intercept
▶	2	0.031	1
	3	0.036	-4.8
*			

 Optimization:
 Step size: 3
 Step count: 3
 Use Results Show Graph...

CE优化编辑

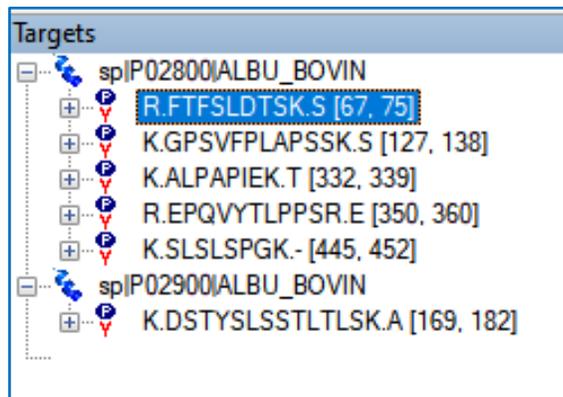
(6) 设置 Peptide Settings 参数。



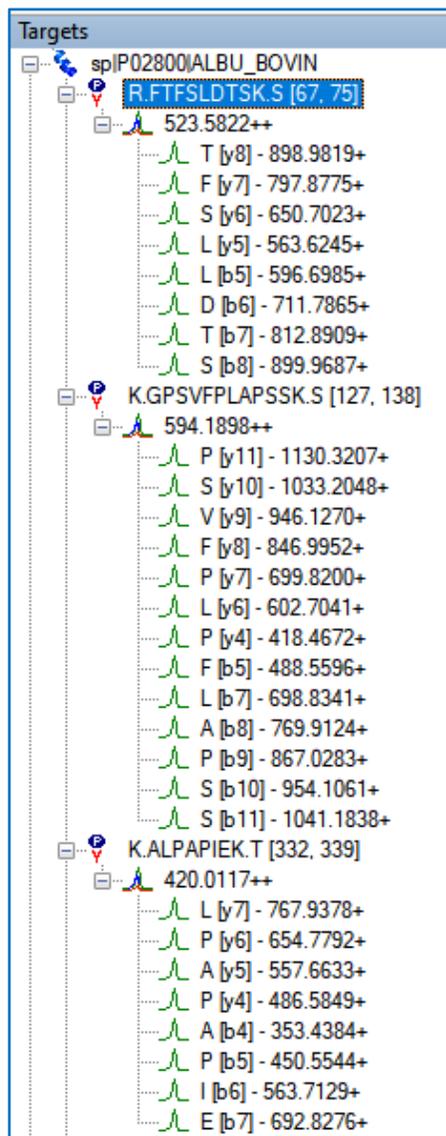
MS 电荷 MSMS离子电荷 离子种类

(6) 设置 Peptide Settings 参数。

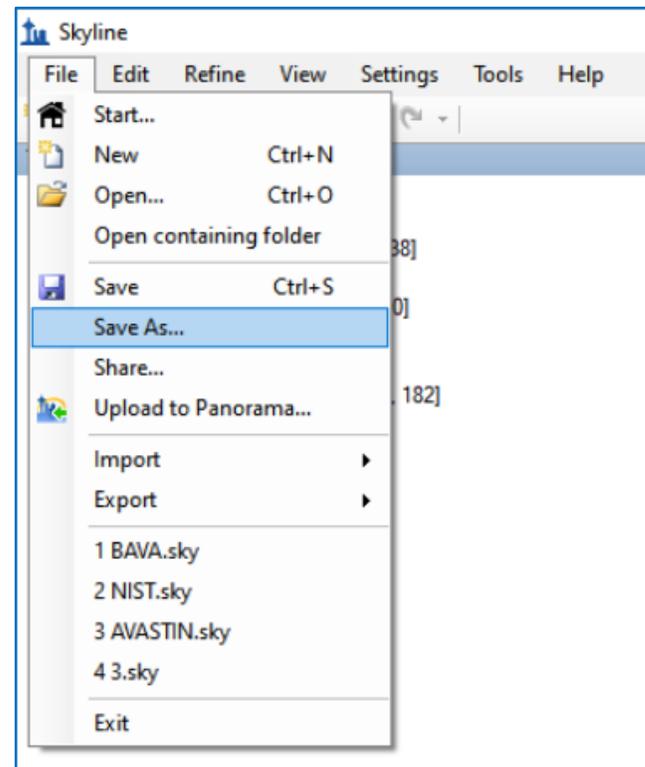
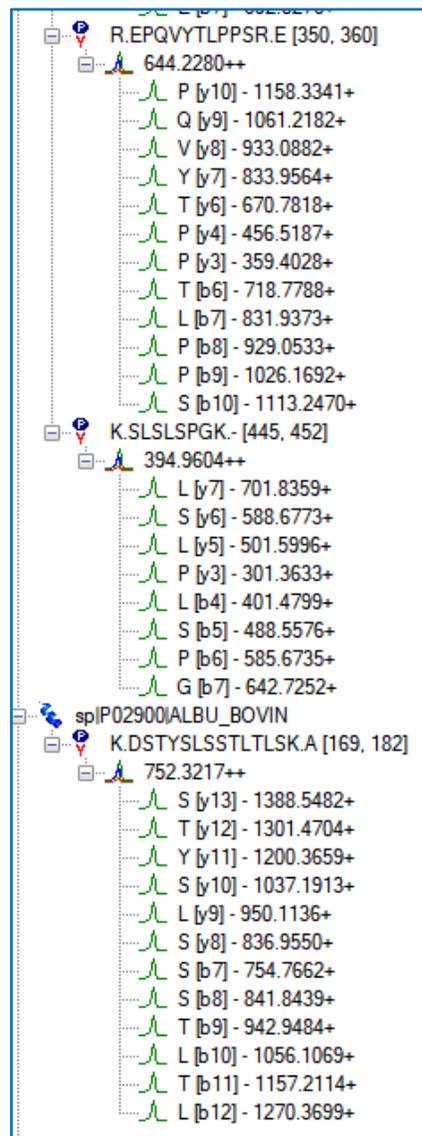
3. 特征肽段



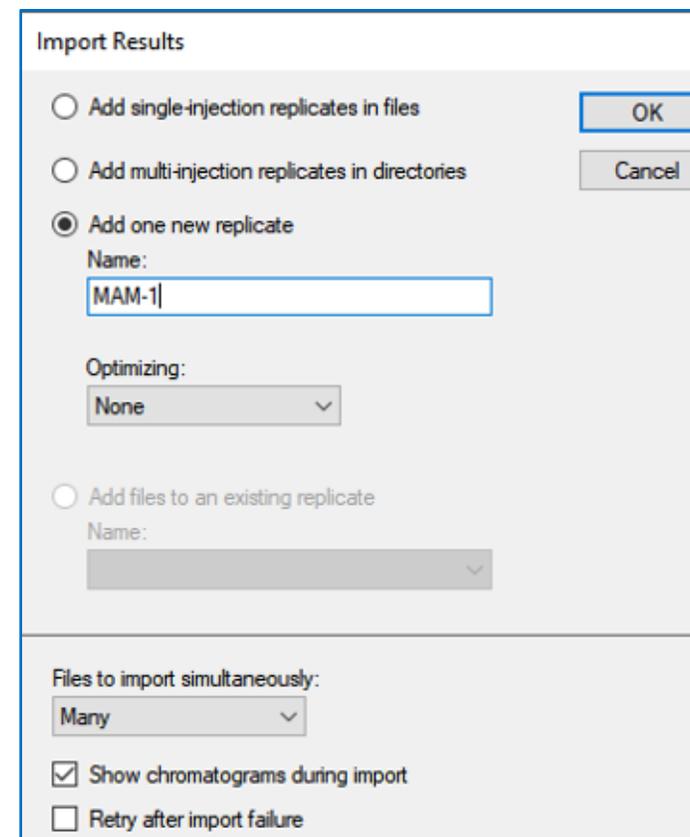
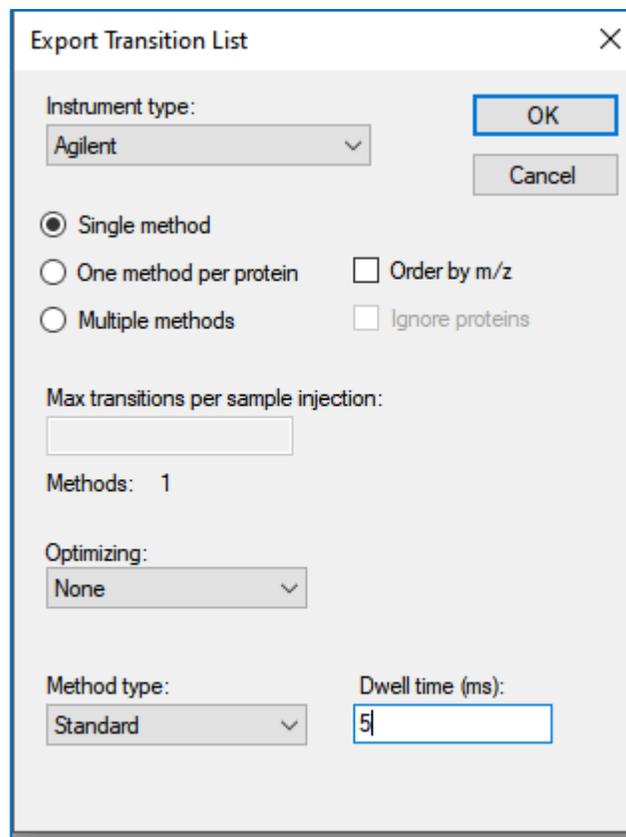
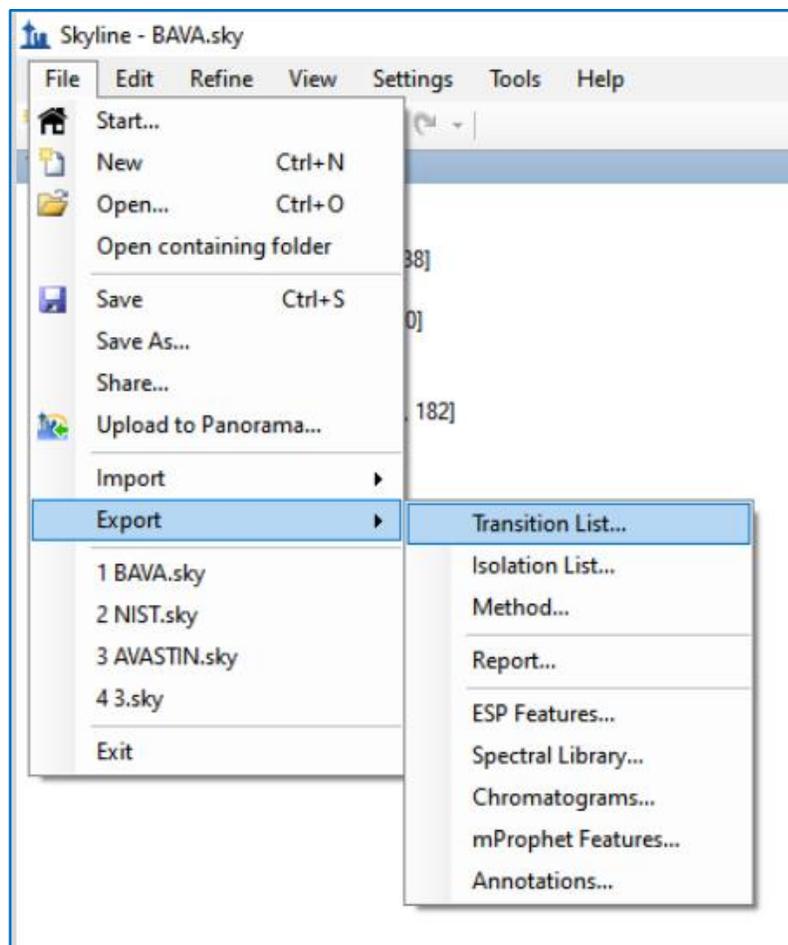
(1) 依据刚才设置的 Peptide Settings 和 Transition Setting 参数,初步筛选出 Avastin 目的肽段;



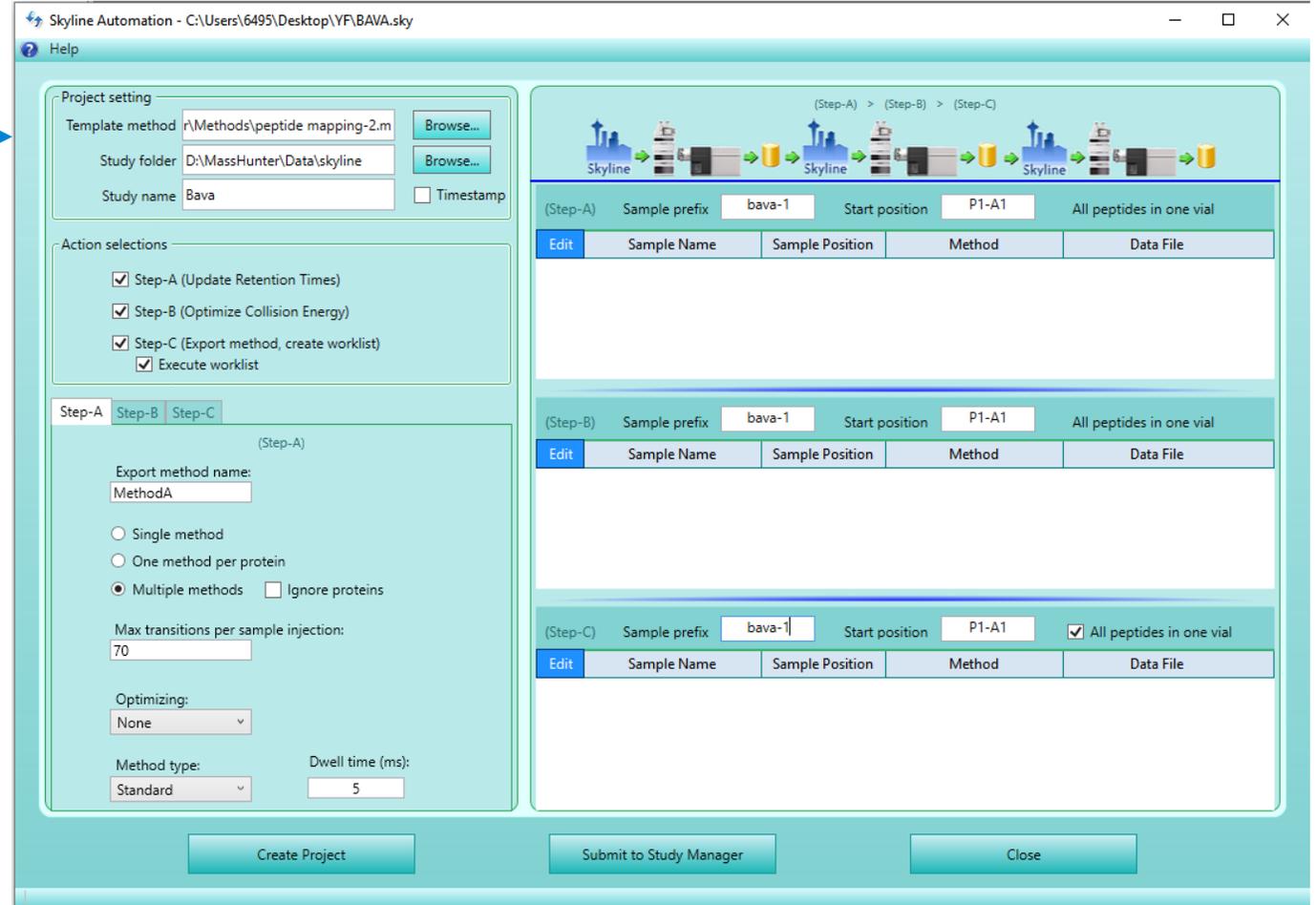
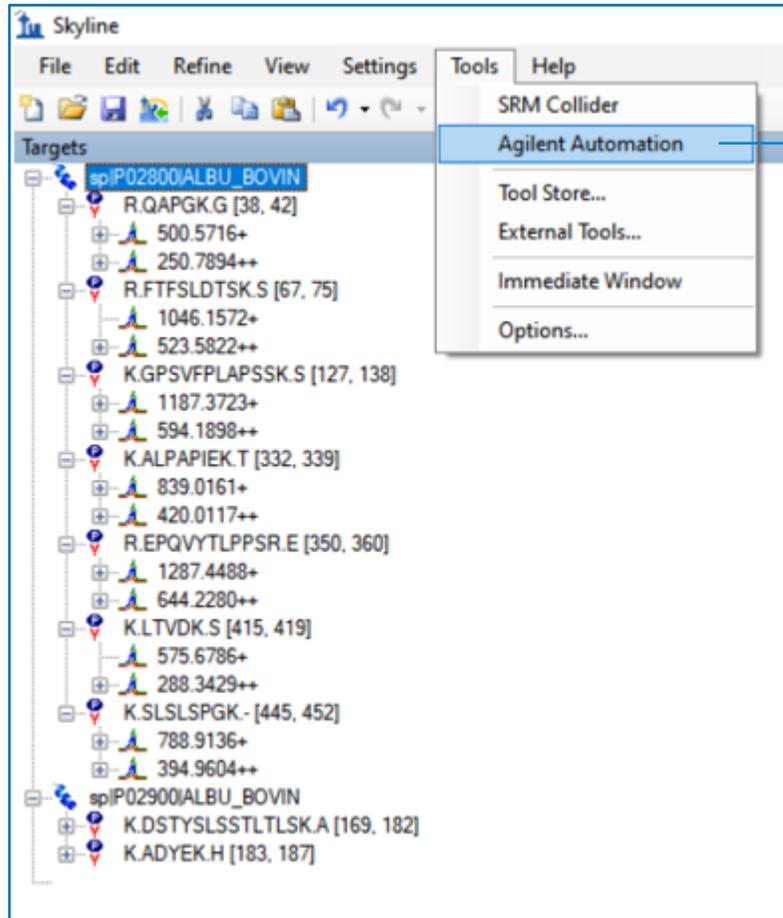
(2) 保存筛选的肽段。



3. Skyline 优化 CE



1. 选取一个肽段，新建，输入肽段，另存为；在Tools 下选择 Automation



Step-A Step-B Step-C

(Step-A)

Export method name:
MethodA

Single method
 One method per protein
 Multiple methods Ignore proteins

Max transitions per sample injection:
70

Optimizing:
None

Method type: Standard Dwell time (ms): 5

Step-A Step-B Step-C

(Step-B)

Export method name:
MethodB

Single method
 One method per protein
 Multiple methods Ignore proteins

Max concurrent transitions:
100

Optimizing:
Collision Energy

Method type: Scheduled

Step-A Step-B Step-C

(Step-C)

Export method name:
MethodC

Single method
 One method per protein
 Multiple methods Ignore proteins

Max concurrent transitions:

Optimizing:
None

Method type: Scheduled

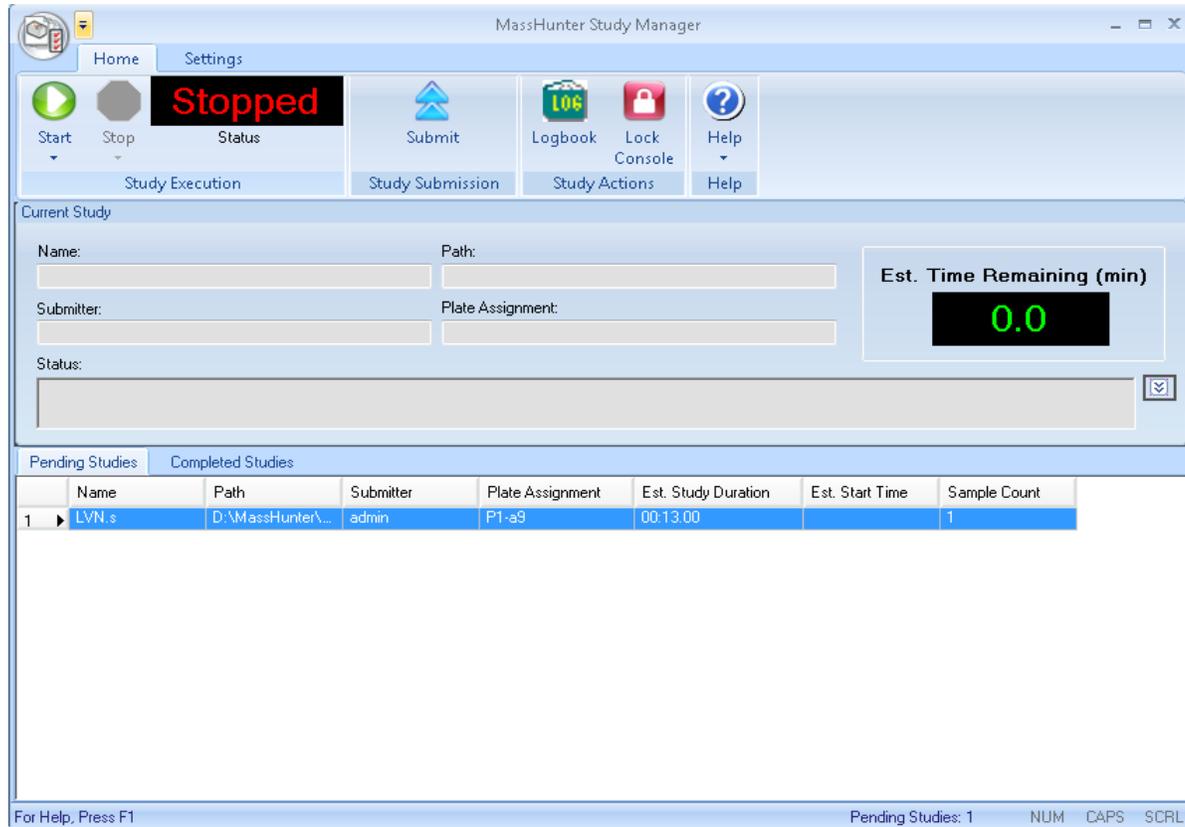
编辑 Step-A, Step-B 和 Step-C 的方法。

设定方法后， Create Project, 优化CE。

The screenshot displays the Skyline Automation software interface. The window title is "Skyline Automation - C:\Users\6495\Desktop\YF\BAVA.sky". The interface is divided into several sections:

- Project setting:** Includes fields for "Template method" (r:\Methods\peptide mapping-2.m), "Study folder" (D:\MassHunter\Data\skyline), and "Study name" (Bava). There are "Browse..." buttons for the first two fields and a "Timestamp" checkbox.
- Action selections:** A list of checkboxes for different steps: Step-A (Update Retention Times), Step-B (Optimize Collision Energy), Step-C (Export method, create worklist), and Execute worklist.
- Step-A configuration:** Shows "Export method name:" as "MethodA". Radio buttons are selected for "Multiple methods" and "Ignore proteins" is unchecked. "Max transitions per sample injection:" is set to "70". "Optimizing:" is set to "None". "Method type:" is "Standard" and "Dwell time (ms):" is "5".
- Workflow visualization:** A top bar shows a sequence of three "Skyline" icons connected by arrows, labeled "(Step-A) > (Step-B) > (Step-C)".
- Step configuration panels:** Three panels are shown for Step-A, Step-B, and Step-C. Each panel has a header with "Sample prefix" (bava-1), "Start position" (P1-A1), and "All peptides in one vial". Below each header is a table with columns: "Sample Name", "Sample Position", "Method", and "Data File". Each panel also has an "Edit" button.
- Bottom navigation:** Three buttons are visible: "Create Project", "Submit to Study Manager", and "Close". These buttons are enclosed in a red dashed box.

Study Manager 启动



1. 选择Start 开始计算;
2. 完成后, 打开Skyline文档并确认导入步骤 A-C 的结果, 确认一切正常
3. 关闭Study Manager和Automation界面, 进入 MussHunter。