

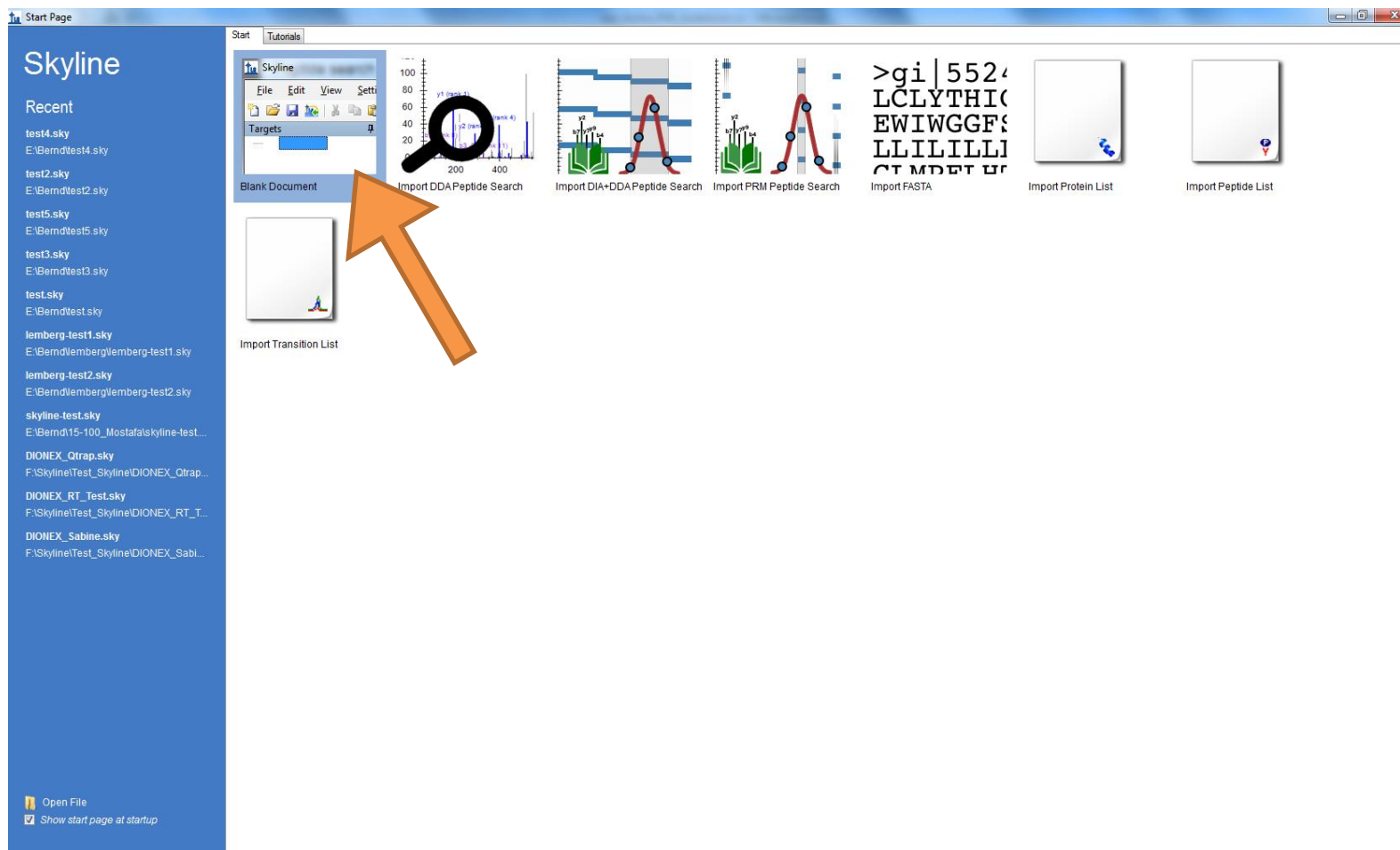
# Creating parent mass lists with skyline

Bernd Hessling

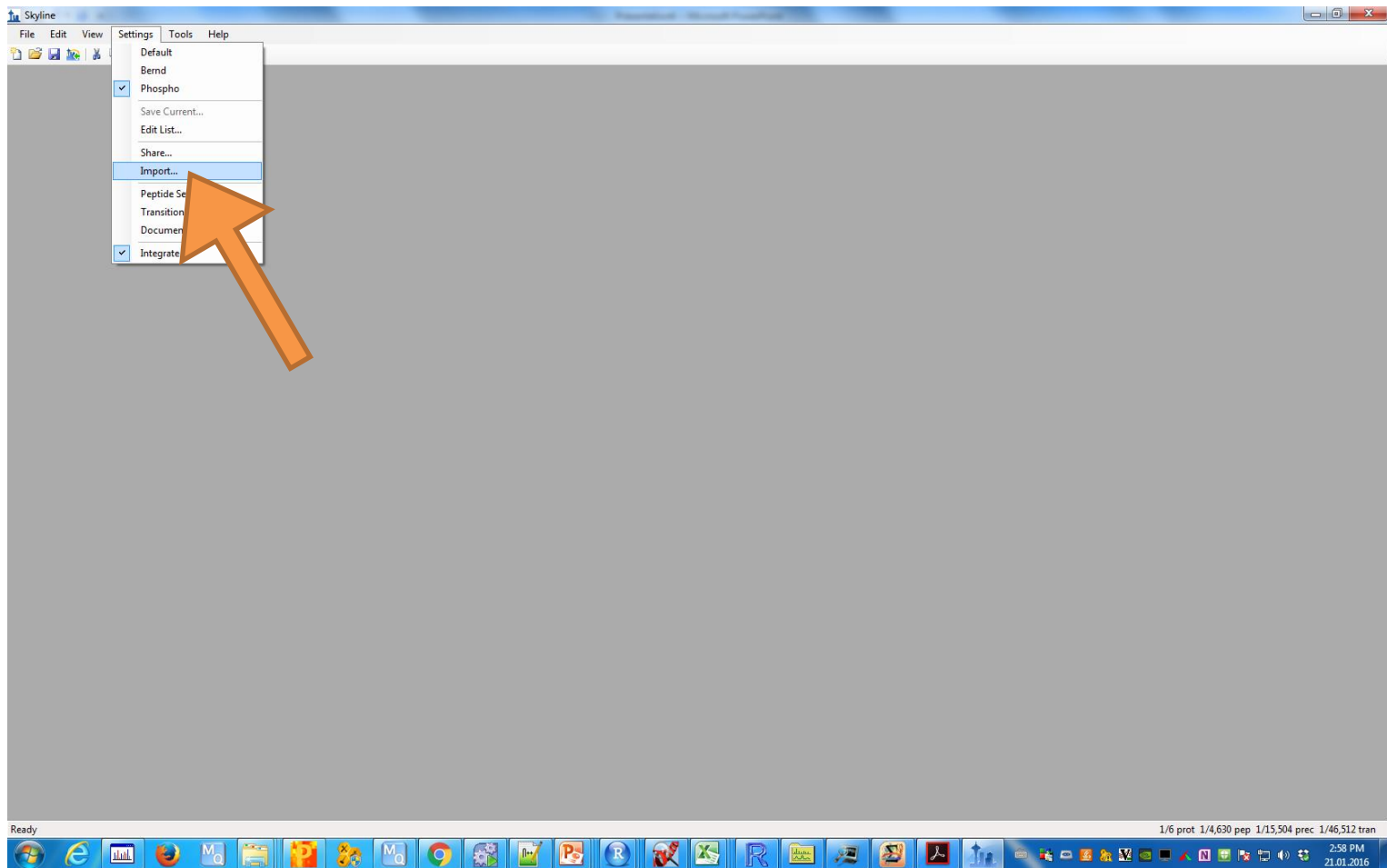
ZMBH

University of Heidelberg

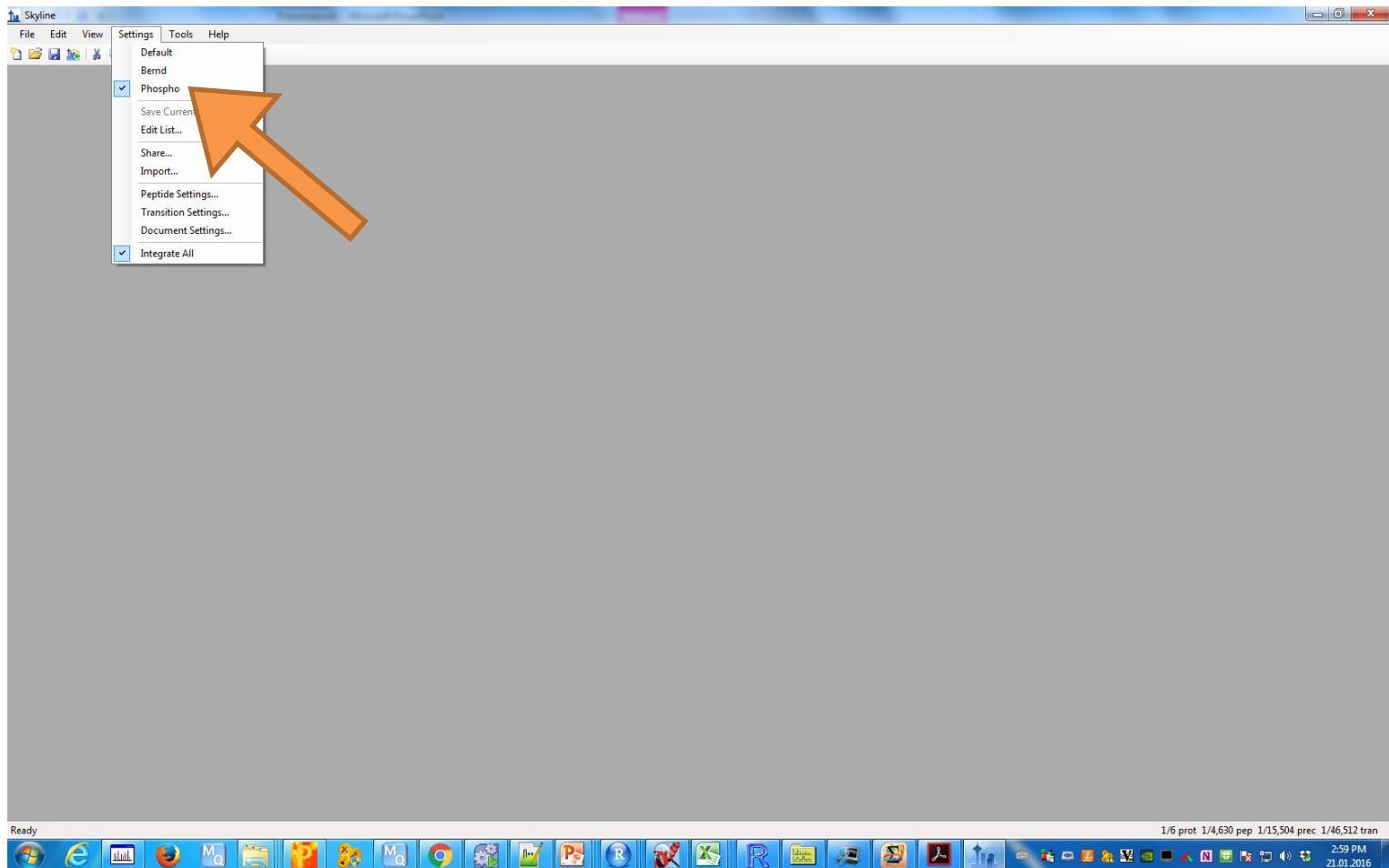
# Open a fresh project in Skyline



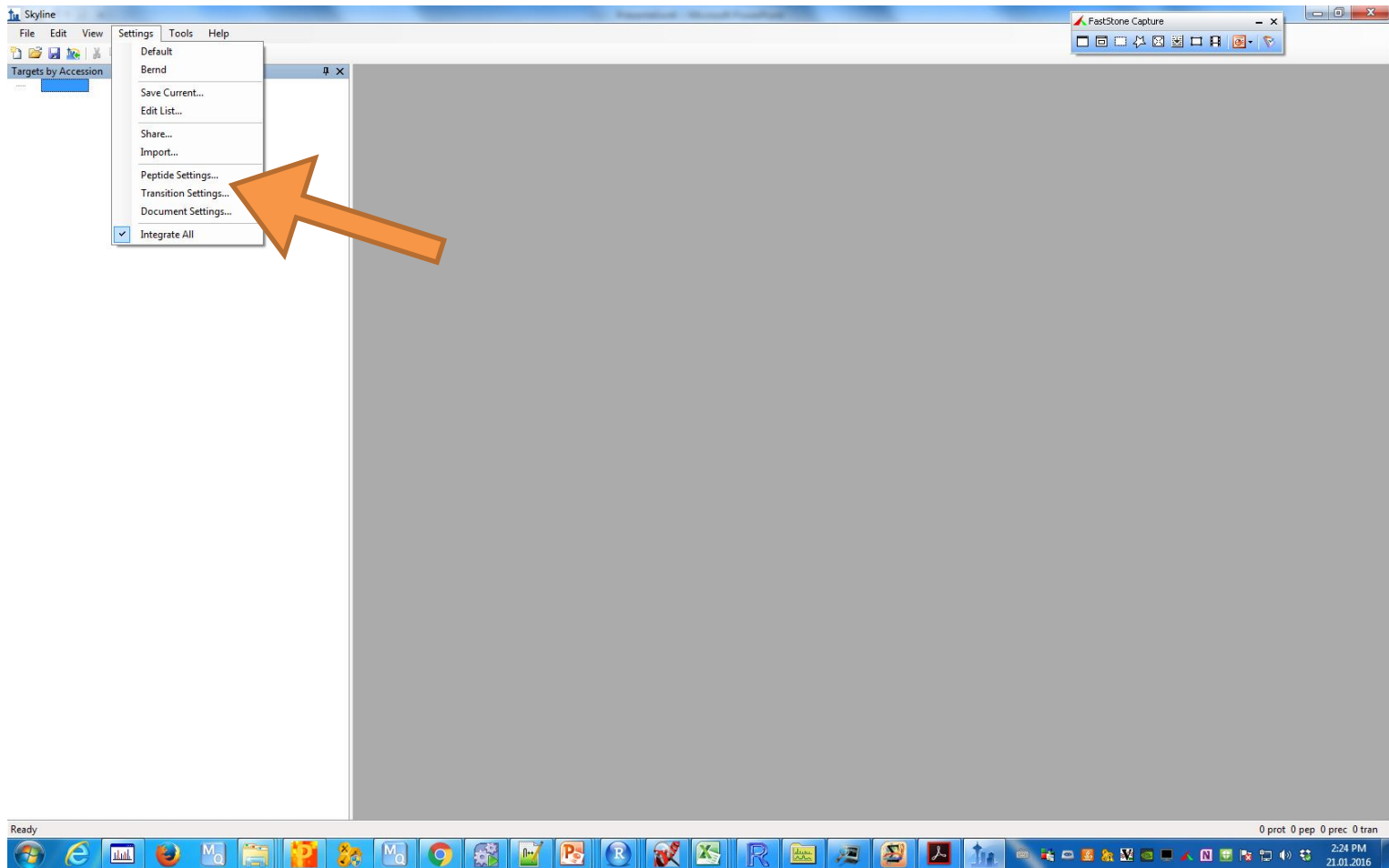
# Import the email attached “Phospho\_Skyline\_Settings.skys”



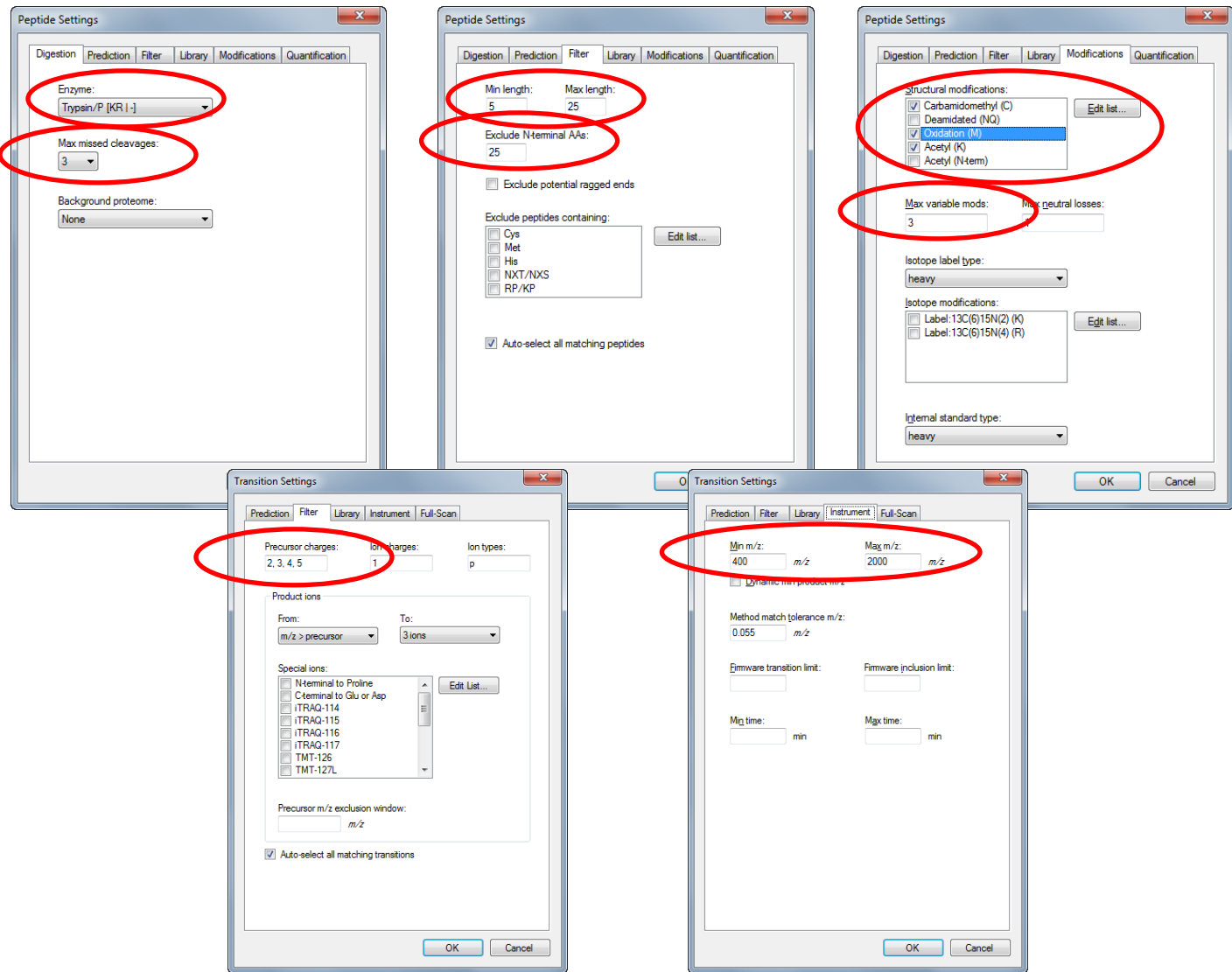
# The default settings for creating Phospho-PMML are loaded



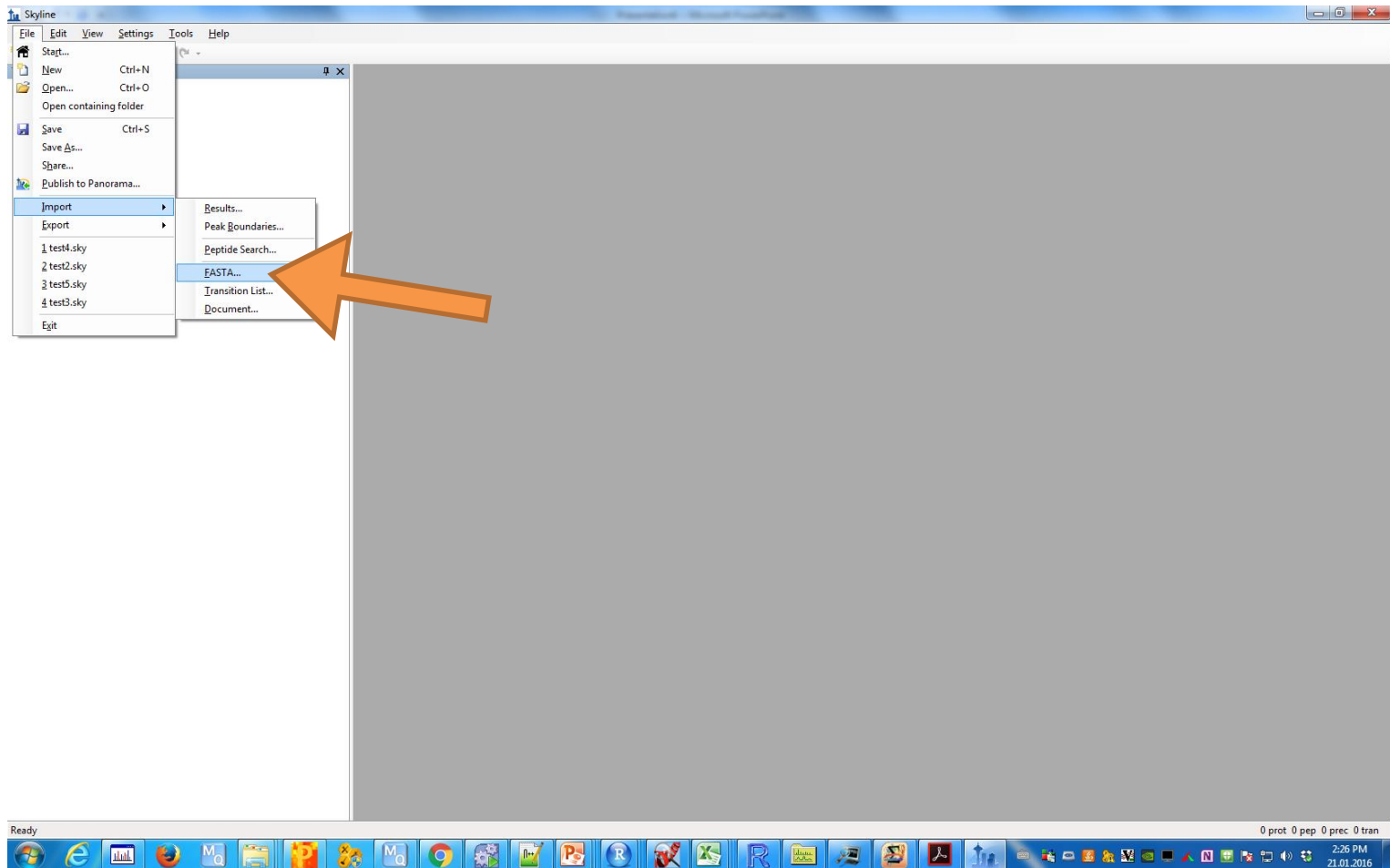
# You can manually check and edit Peptide and transition settings



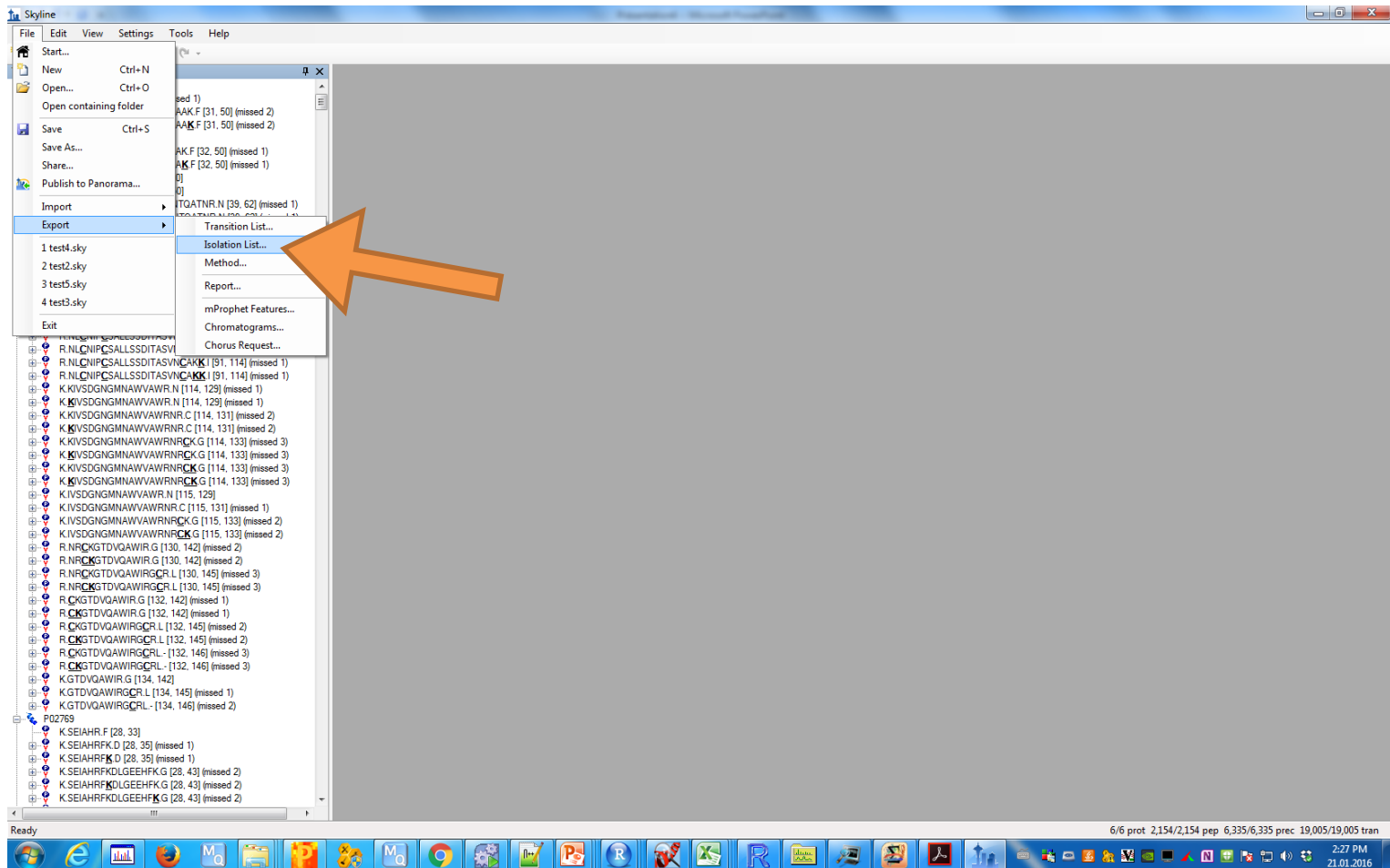
# Here the most important parameter



# Load the FASTA file containing your protein of interest

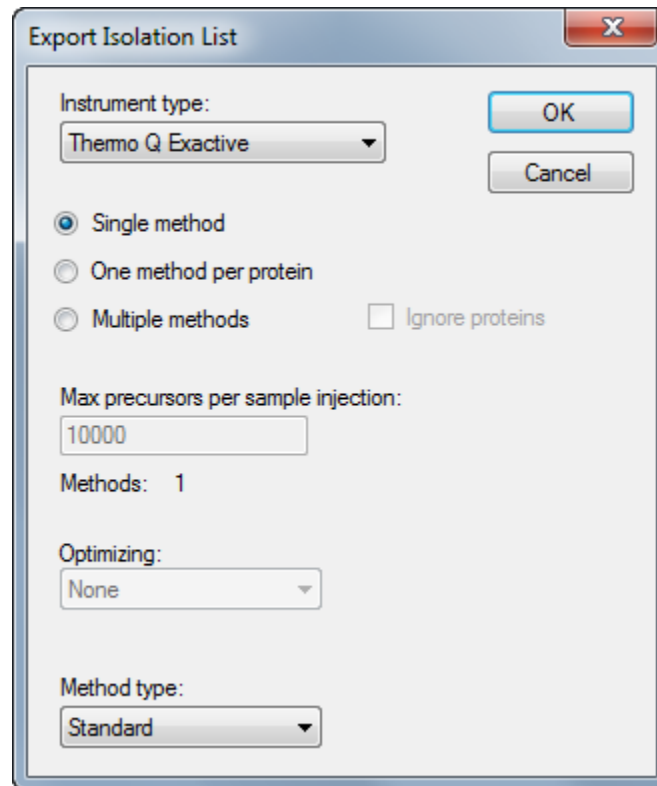


# Export your PML as “Isolation List”...





... and save it for “Instrument type”  
“Thermo Q Exactive”



The image shows a software dialog box titled "Export Isolation List". It contains several configuration options for exporting data. The "Instrument type" is set to "Thermo Q Exactive". Under the "Single method" radio button, the "Ignore proteins" checkbox is unchecked. The "Max precursors per sample injection" is set to 10000. The "Methods" count is 1. The "Optimizing" dropdown is set to "None". The "Method type" is set to "Standard". There are "OK" and "Cancel" buttons in the top right corner.

Export Isolation List

Instrument type:  
Thermo Q Exactive

OK  
Cancel

☒ Single method  
☐ One method per protein  
☐ Multiple methods ☐ Ignore proteins

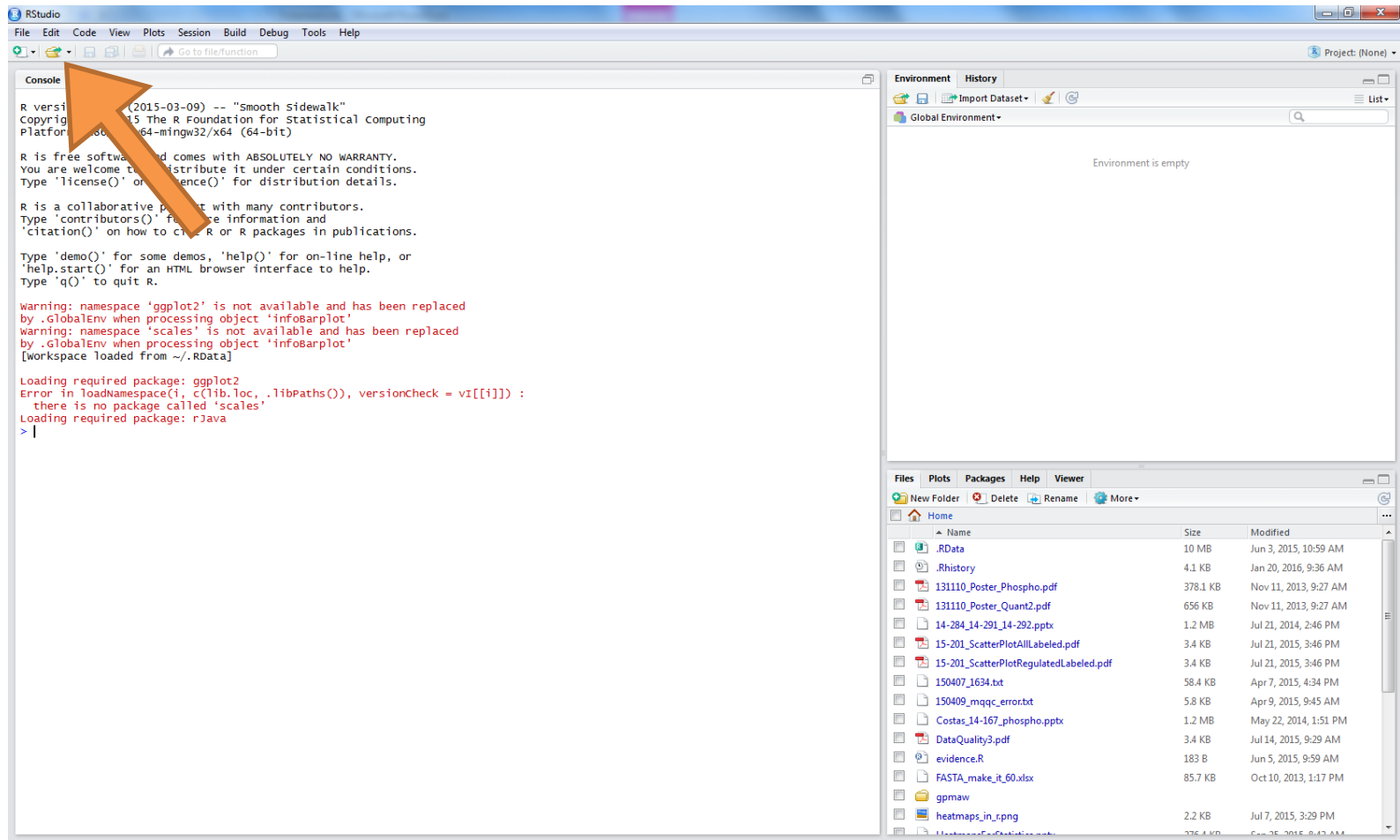
Max precursors per sample injection:  
10000

Methods: 1

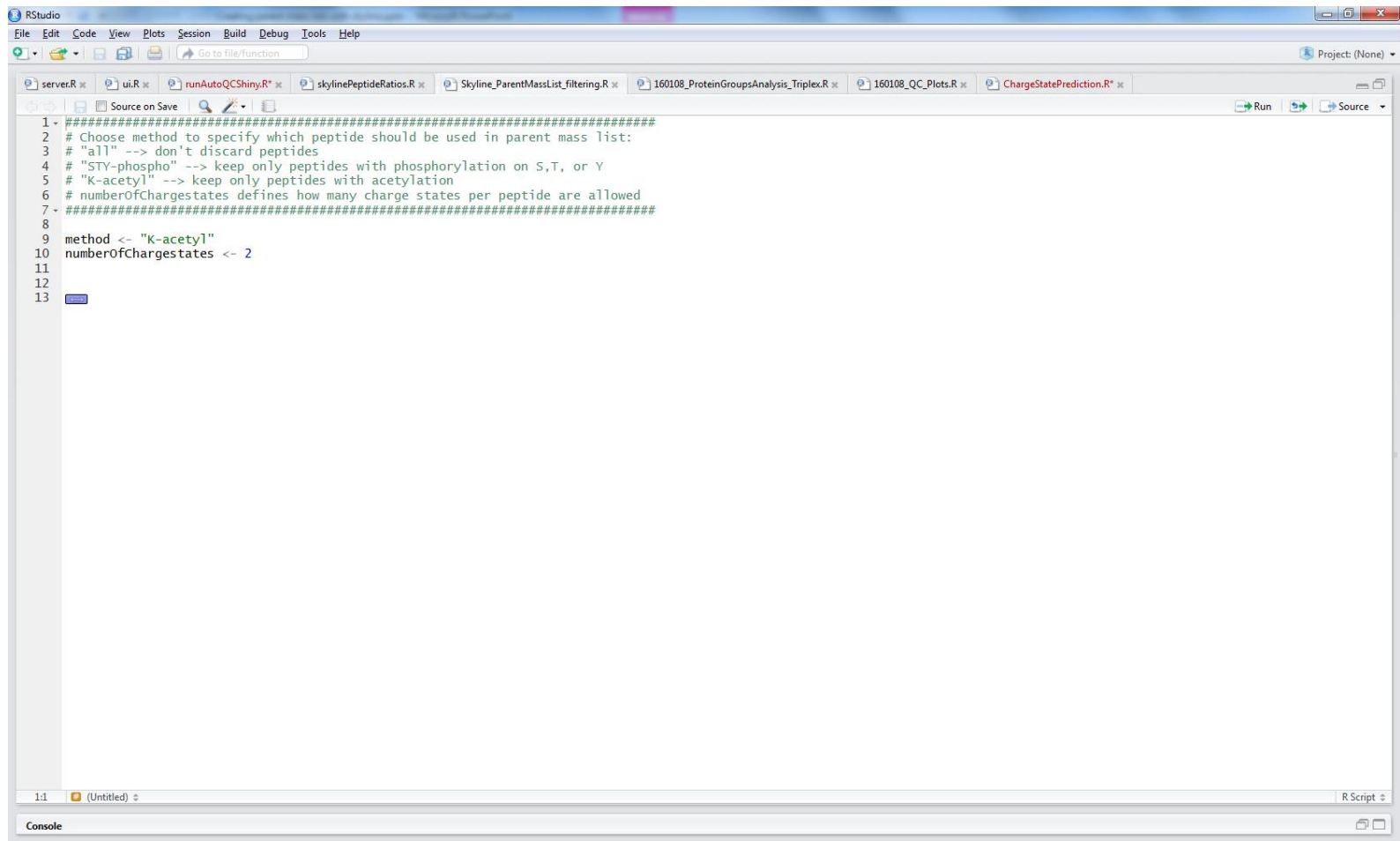
Optimizing:  
None

Method type:  
Standard

# Open RStudio and load the attached script: “Skyline\_ParentMassList\_filtering.R”



# Open RStudio and load the attached script: “Skyline\_ParentMassList\_filtering.R”

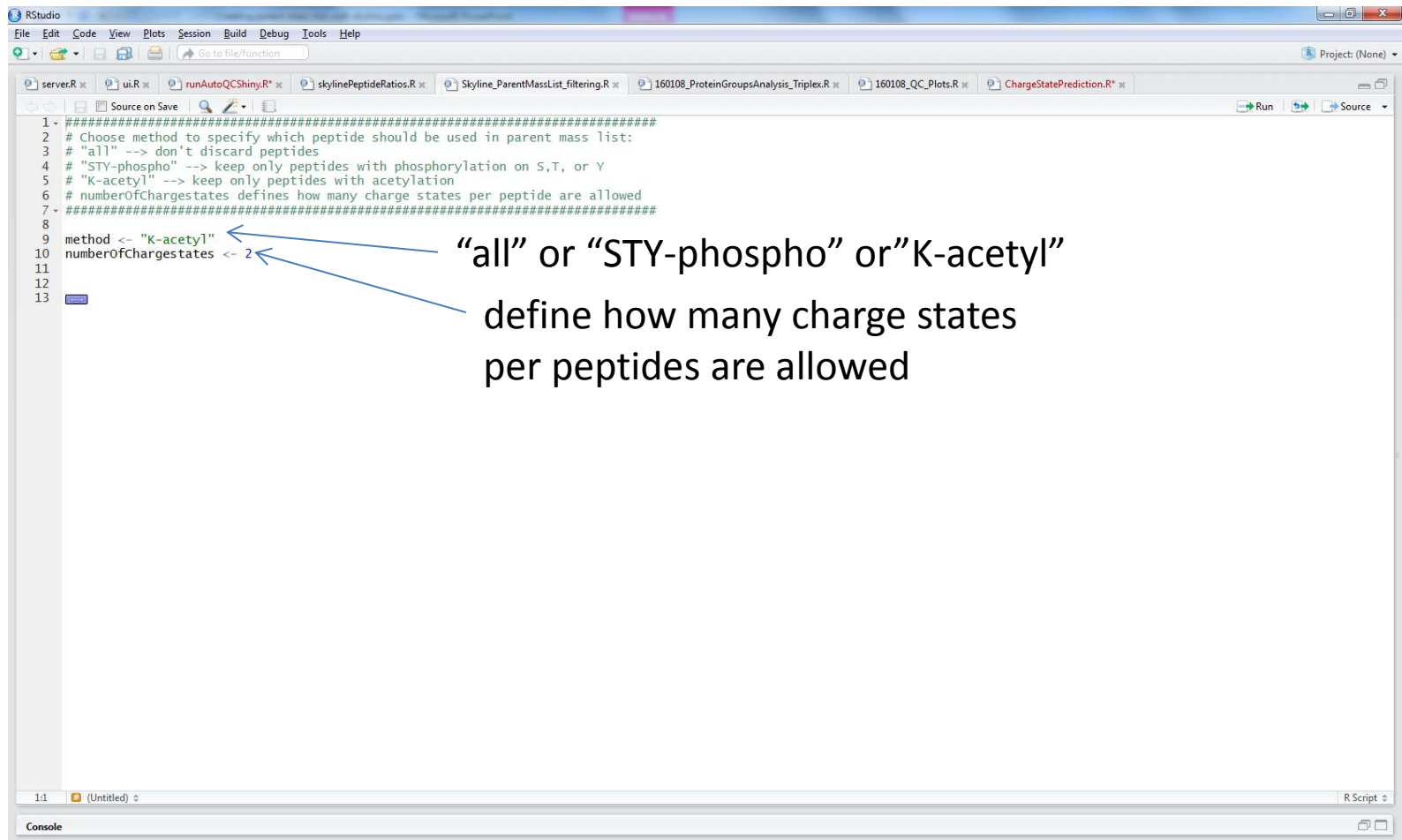


The screenshot shows the RStudio environment with the following details:

- Menu Bar:** File, Edit, Code, View, Plots, Session, Build, Debug, Tools, Help.
- Toolbar:** Includes icons for file operations and a search bar labeled "Go to file/function".
- Project:** Project: (None).
- Source Window:** Contains the script "Skyline\_ParentMassList\_filtering.R". The script content is as follows:

```
1 - #####  
2 # Choose method to specify which peptide should be used in parent mass list:  
3 # "all" --> don't discard peptides  
4 # "STY-phospho" --> keep only peptides with phosphorylation on S,T, or Y  
5 # "K-acetyl" --> keep only peptides with acetylation  
6 # numberOfChargestates defines how many charge states per peptide are allowed  
7 - #####  
8  
9 method <- "K-acetyl"  
10 numberOfChargestates <- 2  
11  
12  
13
```
- Console:** Labeled "Console" at the bottom left.
- Status Bar:** Shows "1:1 (Untitled)" and "R Script".

# Choose the parameters that should be applied to the dataset:



```
1 #####
2 # Choose method to specify which peptide should be used in parent mass list:
3 # "all" --> don't discard peptides
4 # "STY-phospho" --> keep only peptides with phosphorylation on S,T, or Y
5 # "K-acetyl" --> keep only peptides with acetylation
6 # numberOfChargestates defines how many charge states per peptide are allowed
7 #####
8
9 method <- "K-acetyl"
10 numberOfChargestates <- 2
11
12
13
```

“all” or “STY-phospho” or “K-acetyl”

define how many charge states per peptides are allowed

# The Script applies following filtering of data:

- calculate the two most likely charge states for each peptide by sum of H,K,R and only keep these charge states
- delete duplicate m/z entries
- according to specified method:
  - “all” = no further filtering
  - “STY-phospho” = keep only peptides bearing a phosphorylation
  - “K-acetylation” = keep only peptides bearing an acetylation not located at c-terminal lysines
- numberOfChargeStates defines how many chargestates per peptide are allowed:
  - 2: the maximum charge state of a peptide and maximum charge state minus 1
  - 3: the maximum charge state and maximum minus 1 and 2
  - ...

# Output file

- output file is stored in same location as input file
- can be directly imported into Q-Exactive
- for other instruments m/z values need to be copied manually