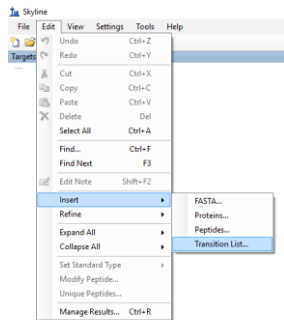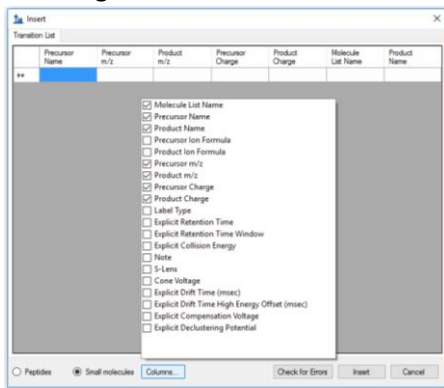Text S1. Protocol for using Skyline for cross-linked peptides

1. Open Skyline and create a blank document. Open the transition list:

2. Select the small molecules option and set up the columns in the transition list table in the following order:

3. Open the tab-delimited text file output from ReACT2prm with a text editor (we recommend Notepad++, https://notepad-plus-plus.org/) and copy paste the information into the Skyline transition list table then click check for errors:
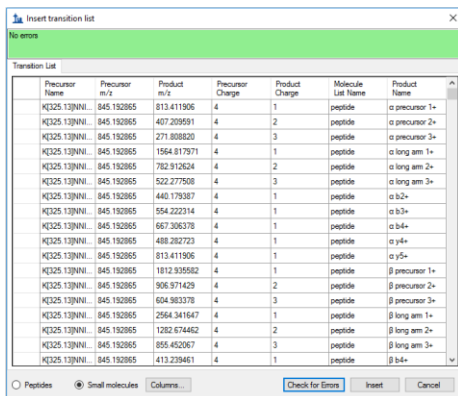
4. Click insert and the transitions for the cross-linked peptide pairs will be uploaded into Skyline:

Skyline

File　Edit　View　Settings　Tools　Help

Targets

- peptide
  - K[325.13]NNIK_ADLINNLGTIAK[325.13]SGTK
    - 845.1929++++
      - α b2+ - 440.1794+
      - α b3+ - 554.2223+
      - α b4+ - 667.3064+
      - α long arm 1+ - 1564.8180+
      - α long arm 2+ - 782.9126++
      - α long arm 3+ - 522.2775+++
      - α precursor 1+ - 813.4119+
      - α precursor 2+ - 407.2096++
      - α precursor 3+ - 271.8088+++
      - α y4+ - 488.2827+
      - α y5+ - 813.4119+
      - β b10+ - 1025.5626+
      - β b11+ - 1096.5997+
      - β b12+ - 1421.7289+
      - β b13+ - 1508.7609+
      - β b14+ - 1565.7824+
      - β b15+ - 1666.8301+
      - β b4+ - 413.2395+
      - β b5+ - 527.2824+
      - β b6+ - 641.3253+
      - β b7+ - 754.4094+
      - β b8+ - 811.4308+
      - β b9+ - 912.4785+
      - β long arm 1+ - 2564.3416+
      - β long arm 2+ - 1282.6745++
      - β long arm 3+ - 855.4521+++
      - β precursor 1+ - 1812.9356+
      - β precursor 2+ - 906.9714++
      - β precursor 3+ - 604.9834+++
      - β y10+ - 1172.6175+
      - β y11+ - 1286.6605+
      - β y12+ - 1400.7034+
      - β y13+ - 1513.7875+
      - β y14+ - 1626.8715+
      - β y15+ - 1741.8985+
      - β y5+ - 717.3432+
      - β y6+ - 788.3803+
      - β y7+ - 901.4643+
      - β y8+ - 1002.5120+
      - β y9+ - 1059.5335+
  - K[333.14]NNIK[136.11]_ADLINNLGTIAK[333.14]SGTK[136.11]
  - ELISNASDALDK[325.13]IR_ADLINNLGTIAK[325.13]SGTK
  - ELISNASDALDK[333.14]IR[162.12]_ADLINNLGTIAK[333.14]SGTK[136.11]
  - ELISNASDALDK[325.13]IR_ADLINNLGTIAK[325.13]SGTK
  - ELISNASDALDK[333.14]IR[162.12]_ADLINNLGTIAK[333.14]SGTK[136.11]
  - NNIK[325.13]LYVR_ADLINNLGTIAK[325.13]SGTK
  - NNIK[333.14]LYVR[162.12]_ADLINNLGTIAK[333.14]SGTK[136.11]
  - FYEAFSK[325.13]NLK_ADLINNLGTIAK[325.13]SGTK
  - FYEAFSK[333.14]NLK[136.11]_ADLINNLGTIAK[333.14]SGTK[136.11]
  - FYEAFSK[325.13]NLK_FYEAFSK[325.13]NLK
  - FYEAFSK[333.14]NLK[136.11]_FYEAFSK[333.14]NLK[136.11]
  - FYEAFSK[325.13]NLK_FYEQFSK[325.13]NIK
  - FYEAFSK[333.14]NLK[136.11]_FYEQFSK[333.14]NIK[136.11]
  - FYEQFSK[325.13]NIK_FYEQFSK[325.13]NIK
  - FYEQFSK[333.14]NIK[136.11]_FYEQFSK[333.14]NIK[136.11]

Ready

5. To export an isolation list for building a PRM method click file->export->isolation list, select the instrument type to be used and click OK:
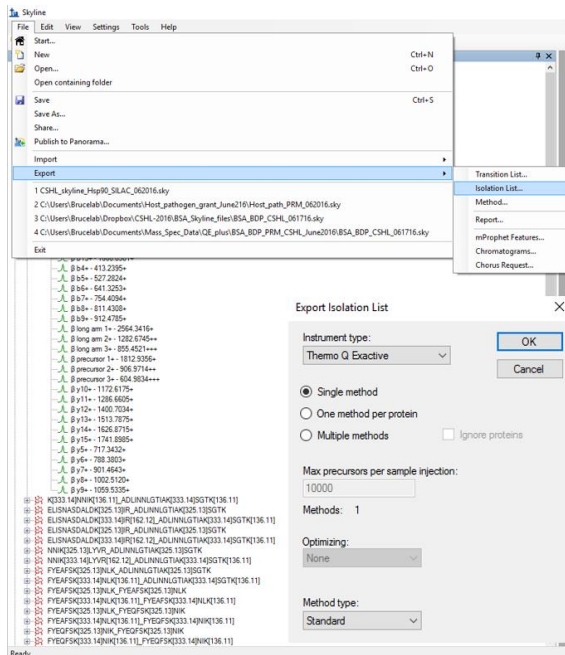
6. At this point the Skyline document can be saved. After collection of PRM mass spectral data Skyline can be used to analyze the data by importing the raw files: