Spec2Seq Manual

by T. Schwaar[a], D. Remmler[a,b], M. G. Weller[a] and H. G. Börner[b]

[a] Bundesanstalt für Materialforschung und -prüfung (BAM)
Division 1.5 Protein Analysis
Richard-Willstätter-Str. 11, 12489 Berlin, Germany

[b] Humboldt-Universität zu Berlin, Department of Chemistry
Laboratory for Organic Synthesis of Functional Systems
Brook-Taylor-Str. 2, 12489 Berlin, Germany
E-mail: h.boerner@hu-berlin.de

Introduction

Spec2Seq offers the opportunity to translate precision polymer fragment spectra into their corresponding monomer sequences. It is especially designed for the analysis of precision polymer libraries, but can also be adapted to different systems by providing a tool for creating a personal monomer block library. Furthermore, it can be used to assign peaks directly from known sequences by using the in-built fragment predictor tool. In the next few pages the P*recision Polymer DeNovo Sequencing*, *Monomer Block Library* and *Fragment Predictor* Tool will be explained.

Precision Polymer DeNovo Sequencing

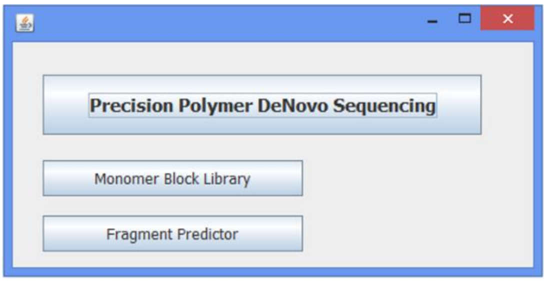
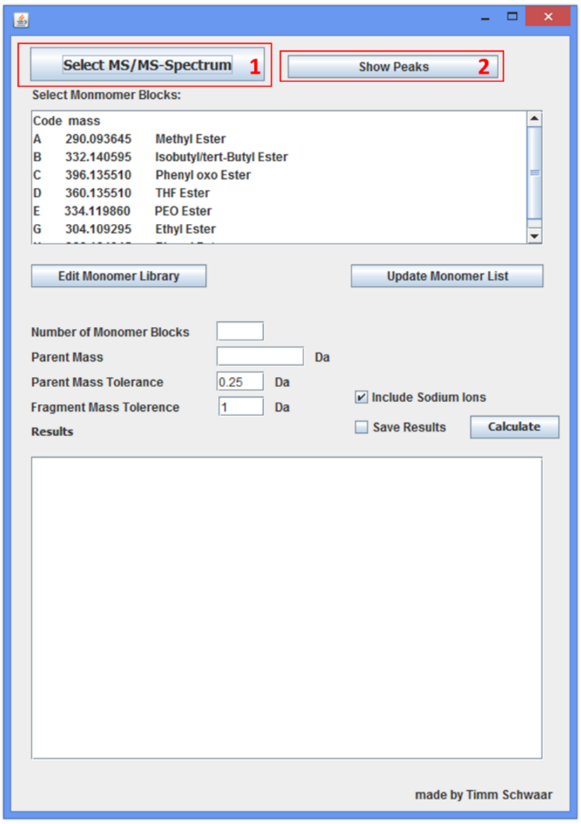


Figure 1. Start the software by double clicking Spec2Seq.jar. The start-screen of Spec2Seq opens, providing the three options *Precision Polymer DeNovo Sequencing*, *Monomer Block Library* and *Fragment Predictor*. Choose *Precision Polymer DeNovo Sequencing*.



Select Monmomer Blocks:

Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester

Number of Monomer Blocks

Parent Mass Da

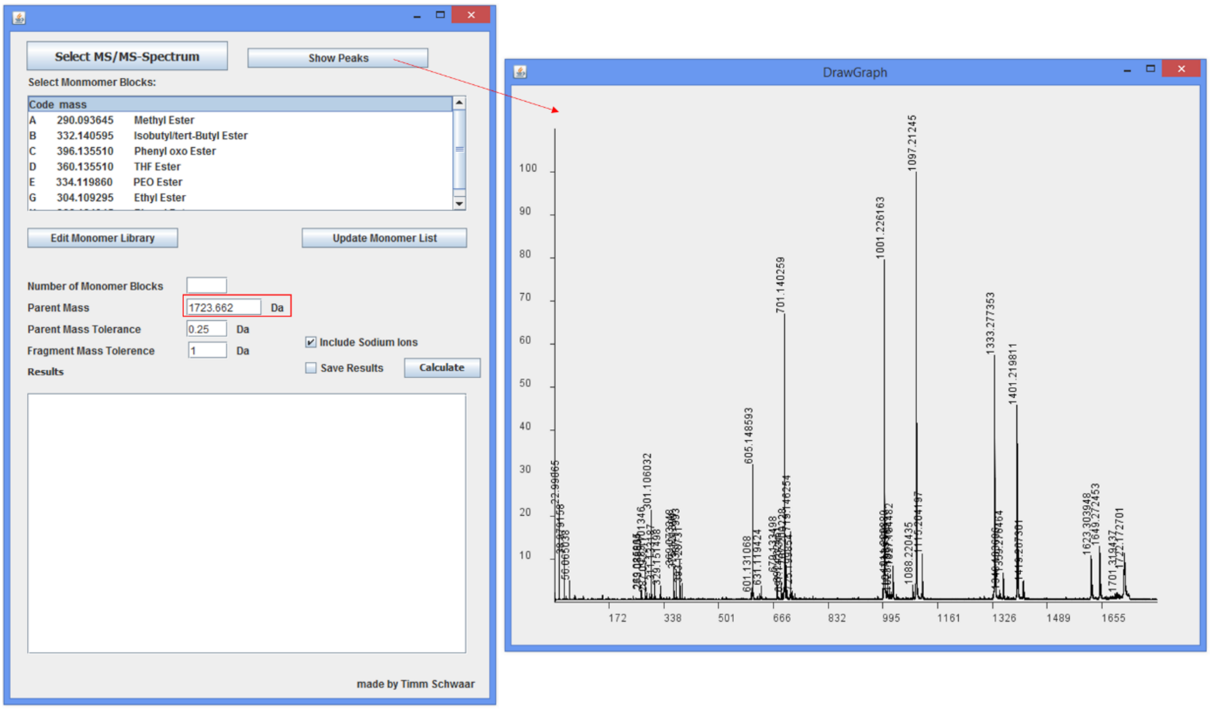
Parent Mass Tolerance 0.25 Da

Fragment Mass Tolerence 1 Da

☑ Include Sodium Ions

☐ Save Results

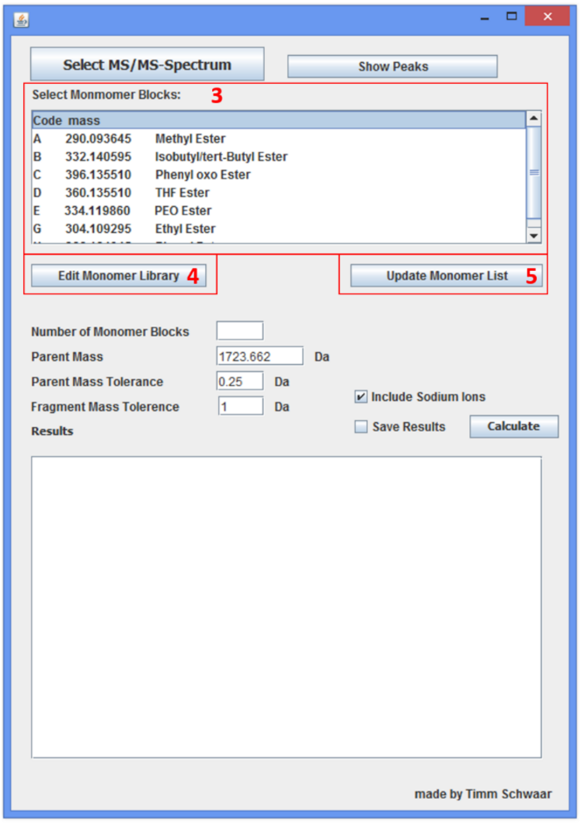
Figure 2. A new interface opens, showing several buttons and options. Start by selecting your ASCII file (e.g. *.txt, *.dat) fragment spectrum file (*Select MS/MS-Spectrum*) and load it into the software (**1**). Click on *Show Peaks* to see all peaks considered for analysis (**2**). The precision polymer A-B-C-G-G was chosen for demonstration.



Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester

Number of Monomer Blocks
Parent Mass 1723.662 Da
Parent Mass Tolerance 0.25 Da
Fragment Mass Tolerence 1 Da
☑ Include Sodium Ions
☐ Save Results
Results

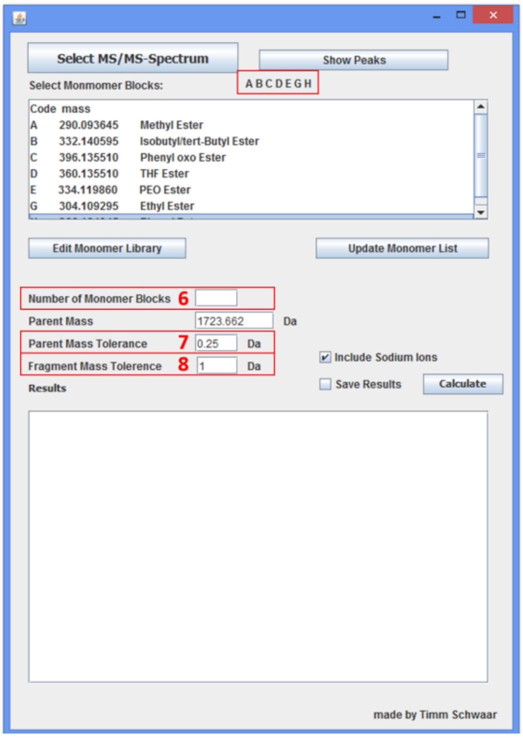
Figure 3. After clicking on *Show Peaks* a second window opens, showing the spectrum and considered peaks for analysis. Note that depending on your ASCII file the *Parent Mass* is already filled with the correct value. If not, please insert manually.



Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester

Number of Monomer Blocks
Parent Mass 1723.662 Da
Parent Mass Tolerance 0.25 Da
Fragment Mass Tolerence 1 Da
☑ Include Sodium Ions
☐ Save Results
Results

Figure 4. The monomer blocks used in the precision polymer library can now be easily selected by double-clicking either on the name, the code or the mass of the monomer (**3**). If your monomer is not yet in the library it can manually be added by clicking *Edit Monomer Library* (**4**). The procedure will be explained in section *Monomer Block Library*. After addition of the newly added monomer block, update the software by clicking *Update Monomer List* (**5**).



Select Monmomer Blocks: A B C D E G H

Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester

Number of Monomer Blocks

Parent Mass 1723.662 Da

Parent Mass Tolerance 0.25 Da

Fragment Mass Tolerence 1 Da

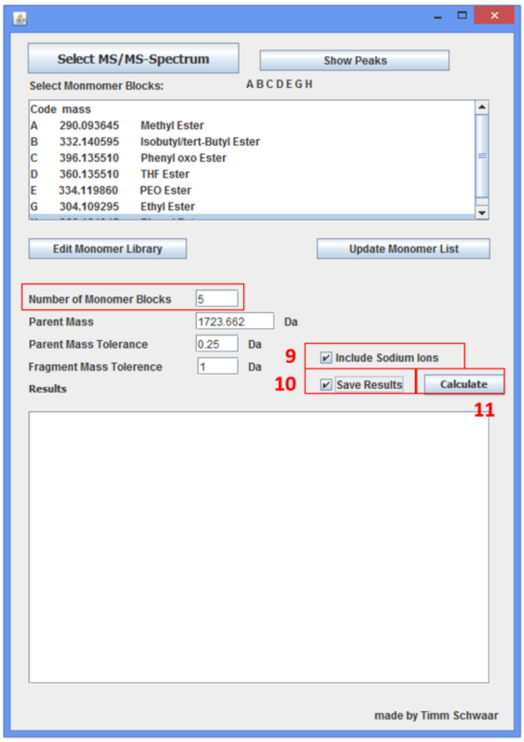
☑ Include Sodium Ions

☐ Save Results

Results

made by Timm Schwaar

Figure 5. The selected monomer blocks now appear next to *Select Monomer Blocks* and can be removed by double-clicking the name of the block in the list. The number of monomer blocks has to be inserted manually (**6**). Default values are already inserted for *Parent Mass Tolerance* and *Fragment Mass Tolerance* and are recommended to use. However, also these values can be edited (**7**) and (**8**).



Select Monmomer Blocks: A B C D E G H

Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester

Number of Monomer Blocks 5

Parent Mass 1723.662 Da

Parent Mass Tolerance 0.25 Da

Fragment Mass Tolerence 1 Da

☑ Include Sodium Ions

☑ Save Results

Results

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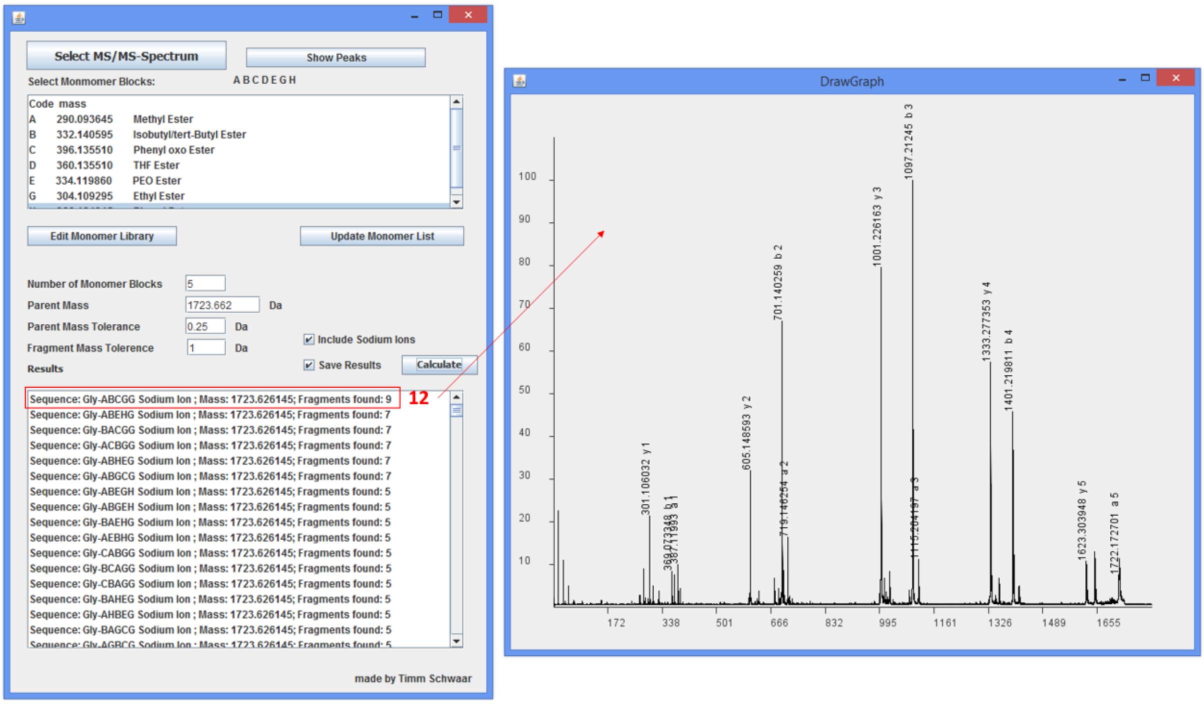
Figure 6. Note that the number of monomer blocks is now filled. For some measurements it is necessary to include sodium ions (**9**), choose only if necessary. The results of the calculations can be saved as *.txt (**10**). Click *Calculate* to start the analysis (**11**).



Select Monmomer Blocks: A B C D E G H

```
Code  mass
A     290.093645     Methyl Ester
B     332.140595     Isobutyl/tert-Butyl Ester
C     396.135510     Phenyl oxo Ester
D     360.135510     THF Ester
E     334.119860     PEO Ester
G     304.109295     Ethyl Ester
```

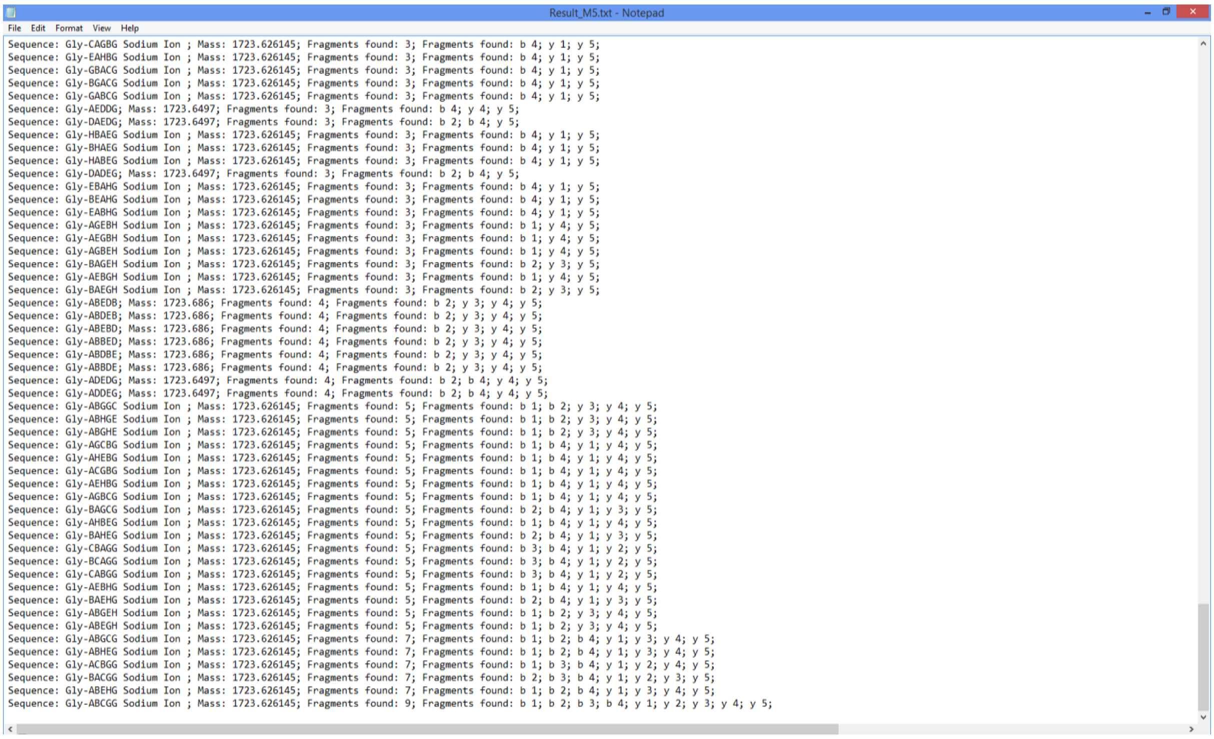
Number of Monomer Blocks: 5
Parent Mass: 1723.662 Da
Parent Mass Tolerance: 0.25 Da
Fragment Mass Tolerence: 1 Da
☑ Include Sodium Ions
☑ Save Results

Results

```
Sequence: Gly-ABCGG Sodium Ion ; Mass: 1723.626145; Fragments found: 9
Sequence: Gly-ABEHG Sodium Ion ; Mass: 1723.626145; Fragments found: 7
Sequence: Gly-BACGG Sodium Ion ; Mass: 1723.626145; Fragments found: 7
Sequence: Gly-ACBGG Sodium Ion ; Mass: 1723.626145; Fragments found: 7
Sequence: Gly-ABHEG Sodium Ion ; Mass: 1723.626145; Fragments found: 7
Sequence: Gly-ABGCG Sodium Ion ; Mass: 1723.626145; Fragments found: 7
Sequence: Gly-ABEGH Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-ABGEH Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-BAEHG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-AEBHG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-CABGG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-BCAGG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-CBAGG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-BAHEG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-AHBEG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-BAGCG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
Sequence: Gly-AGBCG Sodium Ion ; Mass: 1723.626145; Fragments found: 5
```

made by Timm Schwaar

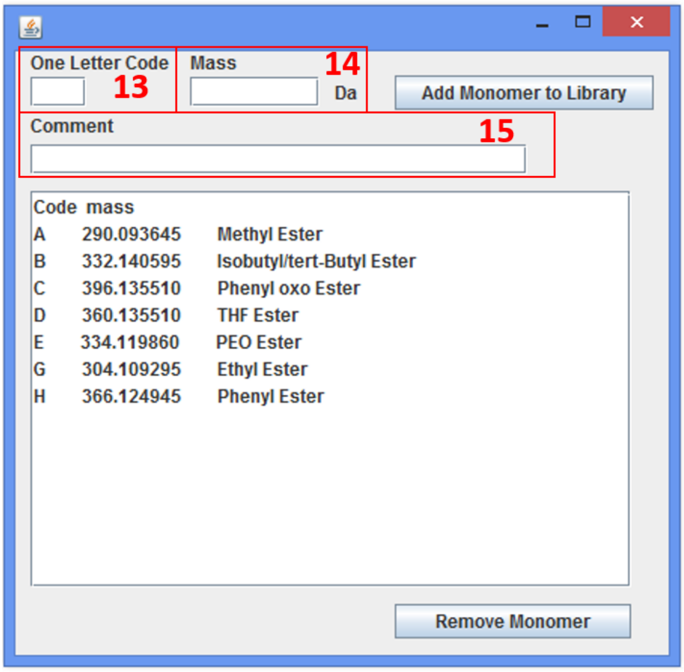
Figure 7. The output of the calculation appear in the result window. They are sorted by the number of fragments found, with the highest number on top. Note that only b- and y-fragments count to the final number. However, the a- and x-fragments are shown in the result spectrum, showing the assigned peaks. It can be opened by double-clicking the name of the sequence (**12**).



```
Sequence: Gly-CAGBG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-EAHBG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-GBACG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-BGACG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-GABCG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-AEDDG; Mass: 1723.6497; Fragments found: 3; Fragments found: b 4; y 4; y 5;
Sequence: Gly-DAEDG; Mass: 1723.6497; Fragments found: 3; Fragments found: b 2; b 4; y 5;
Sequence: Gly-HBAEG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-BHAEG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-HABEG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-DADEG; Mass: 1723.6497; Fragments found: 3; Fragments found: b 2; b 4; y 5;
Sequence: Gly-EBAHG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-BEAHG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-EABHG Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 4; y 1; y 5;
Sequence: Gly-AGEBH Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 1; y 4; y 5;
Sequence: Gly-AEGBH Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 1; y 4; y 5;
Sequence: Gly-AGBEH Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 1; y 4; y 5;
Sequence: Gly-BAGEH Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 2; y 3; y 5;
Sequence: Gly-AEBGH Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 1; y 4; y 5;
Sequence: Gly-BAEGH Sodium Ion ; Mass: 1723.626145; Fragments found: 3; Fragments found: b 2; y 3; y 5;
Sequence: Gly-ABEDB; Mass: 1723.686; Fragments found: 4; Fragments found: b 2; y 3; y 4; y 5;
Sequence: Gly-ABDEB; Mass: 1723.686; Fragments found: 4; Fragments found: b 2; y 3; y 4; y 5;
Sequence: Gly-ABEBD; Mass: 1723.686; Fragments found: 4; Fragments found: b 2; y 3; y 4; y 5;
Sequence: Gly-ABBED; Mass: 1723.686; Fragments found: 4; Fragments found: b 2; y 3; y 4; y 5;
Sequence: Gly-ABDBE; Mass: 1723.686; Fragments found: 4; Fragments found: b 2; y 3; y 4; y 5;
Sequence: Gly-ABBDE; Mass: 1723.686; Fragments found: 4; Fragments found: b 2; y 3; y 4; y 5;
Sequence: Gly-ADEDG; Mass: 1723.6497; Fragments found: 4; Fragments found: b 2; b 4; y 4; y 5;
Sequence: Gly-ADDEG; Mass: 1723.6497; Fragments found: 4; Fragments found: b 2; b 4; y 4; y 5;
Sequence: Gly-ABGGC Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 2; y 3; y 4; y 5;
Sequence: Gly-ABHGE Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 2; y 3; y 4; y 5;
Sequence: Gly-ABGHE Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 2; y 3; y 4; y 5;
Sequence: Gly-AGCBG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 4; y 1; y 4; y 5;
Sequence: Gly-AHEBG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 4; y 1; y 4; y 5;
Sequence: Gly-ACGBG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 4; y 1; y 4; y 5;
Sequence: Gly-AEHBG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 4; y 1; y 4; y 5;
Sequence: Gly-AGBCG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 4; y 1; y 4; y 5;
Sequence: Gly-BAGCG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 2; b 4; y 1; y 3; y 5;
Sequence: Gly-AHBEG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 4; y 1; y 4; y 5;
Sequence: Gly-BAHEG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 2; b 4; y 1; y 3; y 5;
Sequence: Gly-CBAGG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 3; b 4; y 1; y 2; y 5;
Sequence: Gly-BCAGG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 3; b 4; y 1; y 2; y 5;
Sequence: Gly-CABGG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 3; b 4; y 1; y 2; y 5;
Sequence: Gly-AEBHG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 4; y 1; y 4; y 5;
Sequence: Gly-BAEHG Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 2; b 4; y 1; y 3; y 5;
Sequence: Gly-ABGEH Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 2; y 3; y 4; y 5;
Sequence: Gly-ABEGH Sodium Ion ; Mass: 1723.626145; Fragments found: 5; Fragments found: b 1; b 2; y 3; y 4; y 5;
Sequence: Gly-ABGCG Sodium Ion ; Mass: 1723.626145; Fragments found: 7; Fragments found: b 1; b 2; b 4; y 1; y 3; y 4; y 5;
Sequence: Gly-ABHEG Sodium Ion ; Mass: 1723.626145; Fragments found: 7; Fragments found: b 1; b 2; b 4; y 1; y 3; y 4; y 5;
Sequence: Gly-ACBGG Sodium Ion ; Mass: 1723.626145; Fragments found: 7; Fragments found: b 1; b 3; b 4; y 1; y 2; y 4; y 5;
Sequence: Gly-BACGG Sodium Ion ; Mass: 1723.626145; Fragments found: 7; Fragments found: b 2; b 3; b 4; y 1; y 2; y 3; y 5;
Sequence: Gly-ABEHG Sodium Ion ; Mass: 1723.626145; Fragments found: 7; Fragments found: b 1; b 2; b 4; y 1; y 3; y 4; y 5;
Sequence: Gly-ABCGG Sodium Ion ; Mass: 1723.626145; Fragments found: 9; Fragments found: b 1; b 2; b 3; b 4; y 1; y 2; y 3; y 4; y 5;
```

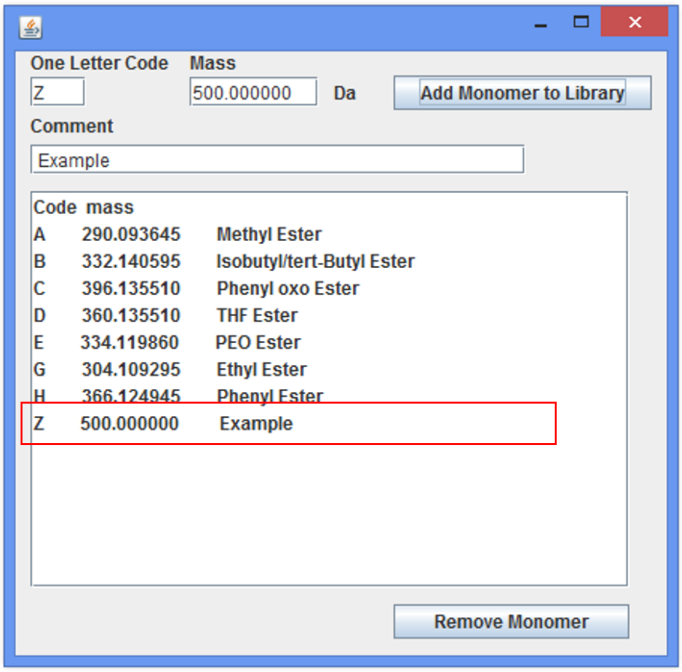
Figure 8. For detailed description such as which fragments were found the result.txt file can be opened.

Monomer Block Library



Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester
H	366.124945	Phenyl Ester

Figure 9. For adding a new monomer to the library a one letter code has to be created (**13**). Note that also numbers are allowed as one letter codes. Next, enter the exact mass of the building block as diradical (**14**). An additional comment can be added such as the full monomer name (**15**). Click *Add Monomer to Library*.

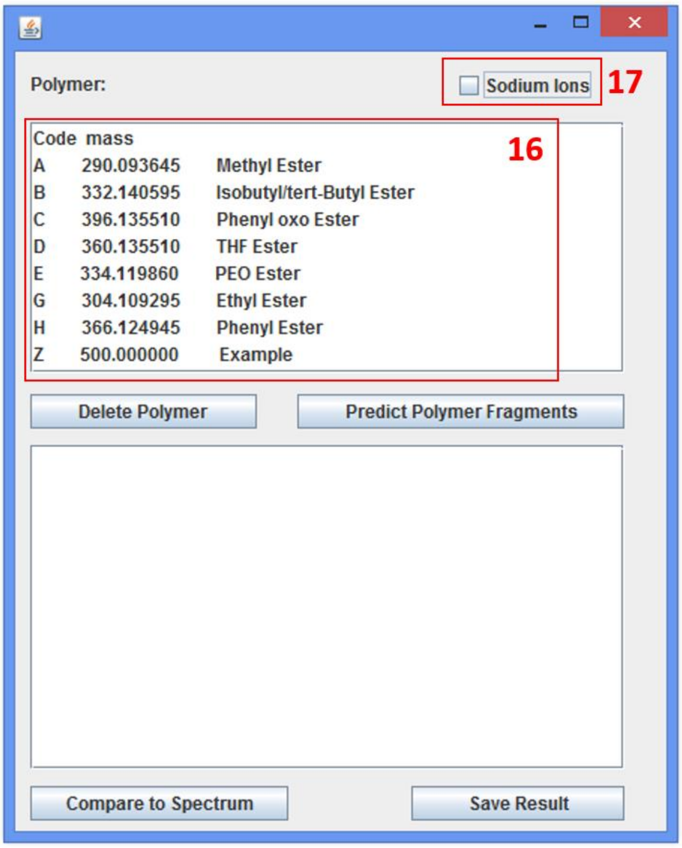


One Letter Code: Z
Mass: 500.000000 Da
Comment: Example

Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester
H	366.124945	Phenyl Ester
Z	500.000000	Example

Figure 10. A monomer block was added with the one letter code “Z”, the mass “500.000000” and the comment “Example”. It appears now in the library.

Fragment Predictor

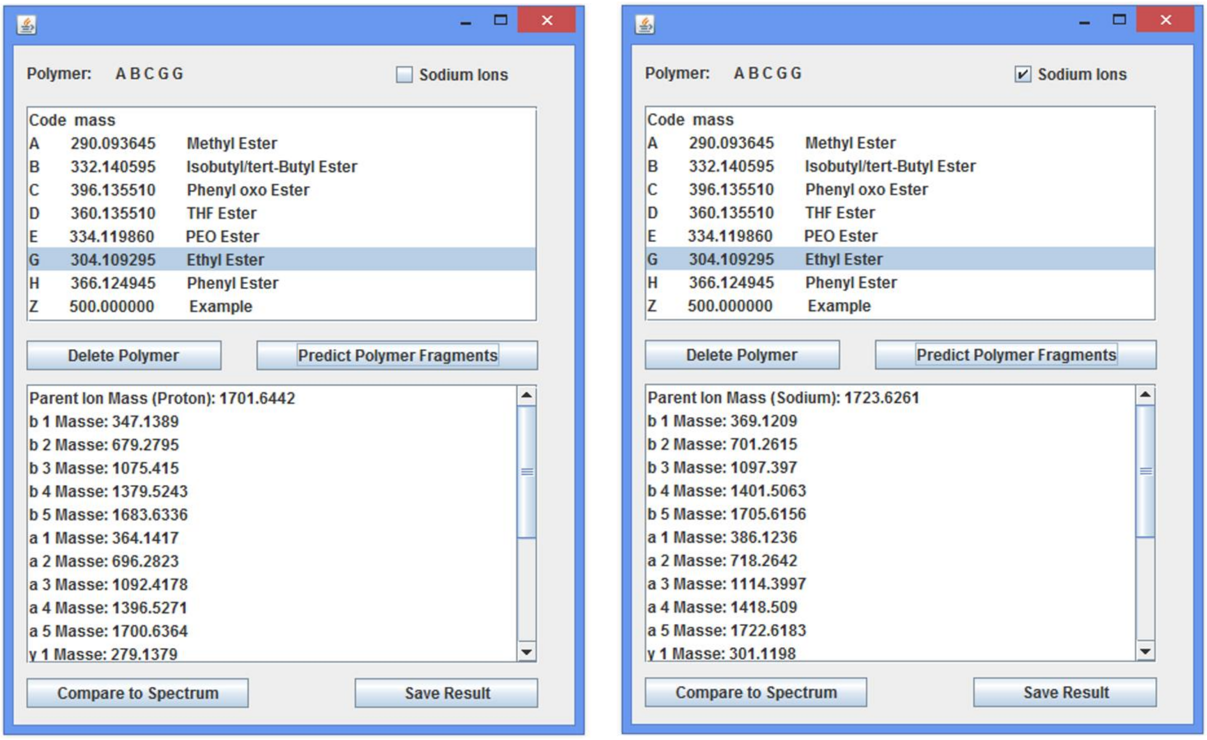


Polymer:

☐ Sodium Ions

```
Code  mass
A     290.093645     Methyl Ester
B     332.140595     Isobutyl/tert-Butyl Ester
C     396.135510     Phenyl oxo Ester
D     360.135510     THF Ester
E     334.119860     PEO Ester
G     304.109295     Ethyl Ester
H     366.124945     Phenyl Ester
Z     500.000000     Example
```

Figure 11. The fragment predictor can be used building up the precision polymer by double clicking the monomers (**16**). It can be chosen to include sodium ion (**17**). By clicking *Predict Polymer Fragments* the software calculates the expected fragments.



Polymer: A B C G G

☐ Sodium Ions

```
Code  mass
A     290.093645     Methyl Ester
B     332.140595     Isobutyl/tert-Butyl Ester
C     396.135510     Phenyl oxo Ester
D     360.135510     THF Ester
E     334.119860     PEO Ester
G     304.109295     Ethyl Ester
H     366.124945     Phenyl Ester
Z     500.000000     Example
```

```
Parent Ion Mass (Proton): 1701.6442
b 1 Masse: 347.1389
b 2 Masse: 679.2795
b 3 Masse: 1075.415
b 4 Masse: 1379.5243
b 5 Masse: 1683.6336
a 1 Masse: 364.1417
a 2 Masse: 696.2823
a 3 Masse: 1092.4178
a 4 Masse: 1396.5271
a 5 Masse: 1700.6364
y 1 Masse: 279.1379
```

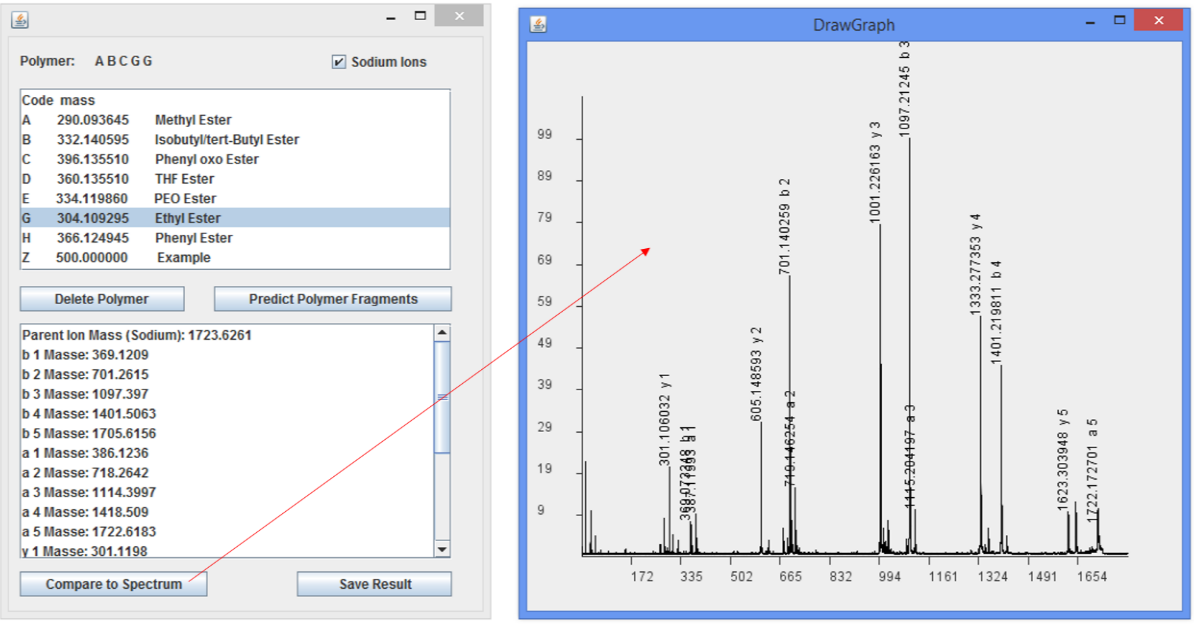
Polymer: A B C G G

☑ Sodium Ions

```
Code  mass
A     290.093645     Methyl Ester
B     332.140595     Isobutyl/tert-Butyl Ester
C     396.135510     Phenyl oxo Ester
D     360.135510     THF Ester
E     334.119860     PEO Ester
G     304.109295     Ethyl Ester
H     366.124945     Phenyl Ester
Z     500.000000     Example
```

```
Parent Ion Mass (Sodium): 1723.6261
b 1 Masse: 369.1209
b 2 Masse: 701.2615
b 3 Masse: 1097.397
b 4 Masse: 1401.5063
b 5 Masse: 1705.6156
a 1 Masse: 386.1236
a 2 Masse: 718.2642
a 3 Masse: 1114.3997
a 4 Masse: 1418.509
a 5 Masse: 1722.6183
y 1 Masse: 301.1198
```

Figure 121. Results of the calculations excluding and including sodium ions.



Polymer: A B C G G

☑ Sodium Ions

Code	mass	
A	290.093645	Methyl Ester
B	332.140595	Isobutyl/tert-Butyl Ester
C	396.135510	Phenyl oxo Ester
D	360.135510	THF Ester
E	334.119860	PEO Ester
G	304.109295	Ethyl Ester
H	366.124945	Phenyl Ester
Z	500.000000	Example

```
Parent Ion Mass (Sodium): 1723.6261
b 1 Masse: 369.1209
b 2 Masse: 701.2615
b 3 Masse: 1097.397
b 4 Masse: 1401.5063
b 5 Masse: 1705.6156
a 1 Masse: 386.1236
a 2 Masse: 718.2642
a 3 Masse: 1114.3997
a 4 Masse: 1418.509
a 5 Masse: 1722.6183
y 1 Masse: 301.1198
```

Figure 13. The button *Compare to spectrum* offers the direct comparison to a measured spectrum. This tool comes in handy for known sequences.

FAQ

1. The result shows “no match”.

   A: This error appears when no sequences can be assigned. Often the parent mass is wrong/missing, or the number of monomer blocks is wrong. Also check whether the *Sodium Ions* button is selected.

2. Length is empty.

   A: Enter a value for the building blocks and try again.

3. No spectrum selected.

   A: The selected spectrum has the wrong format or cannot be read. Only ASCII files (e.g. *.txt, *.dat) accepted.