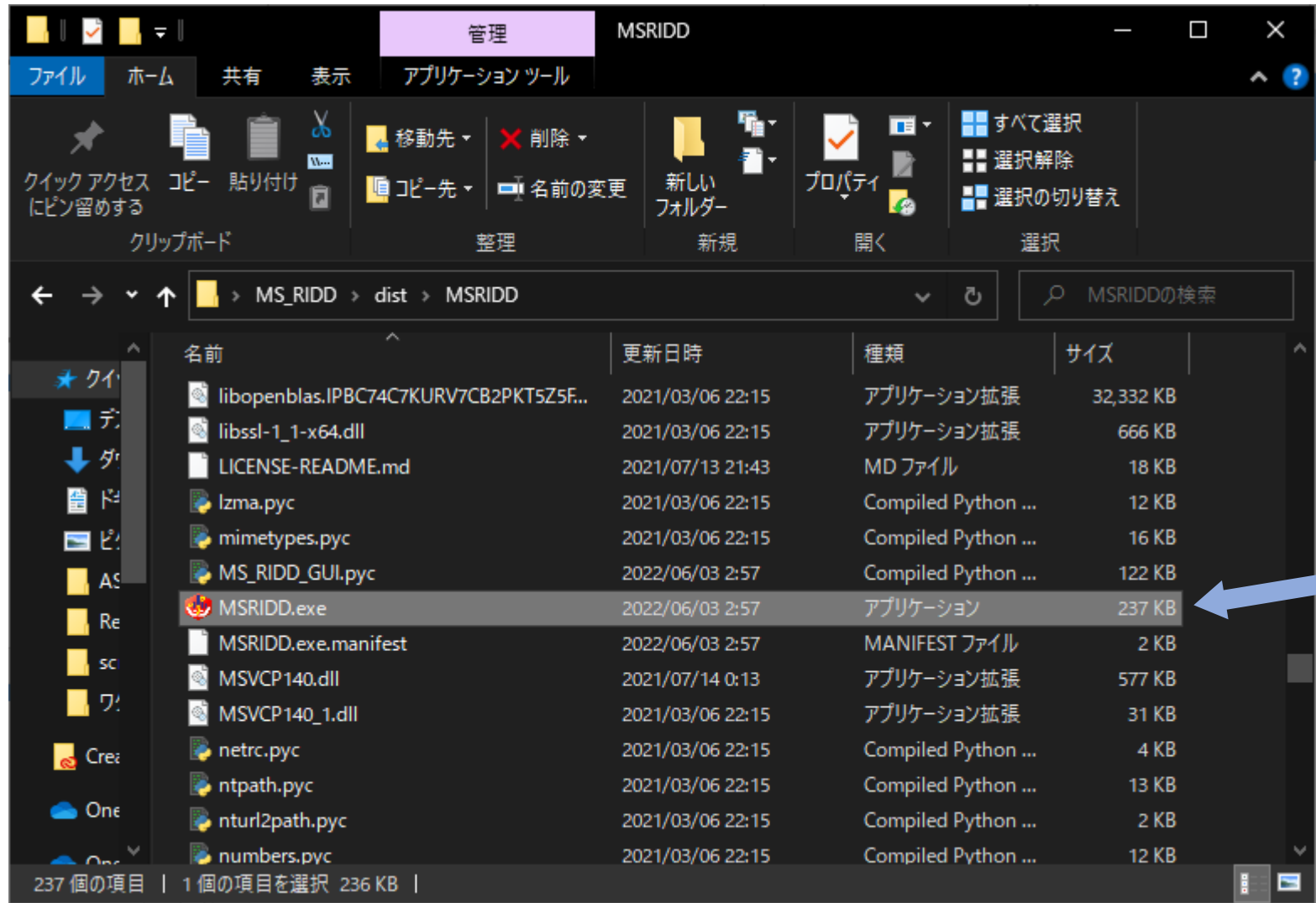


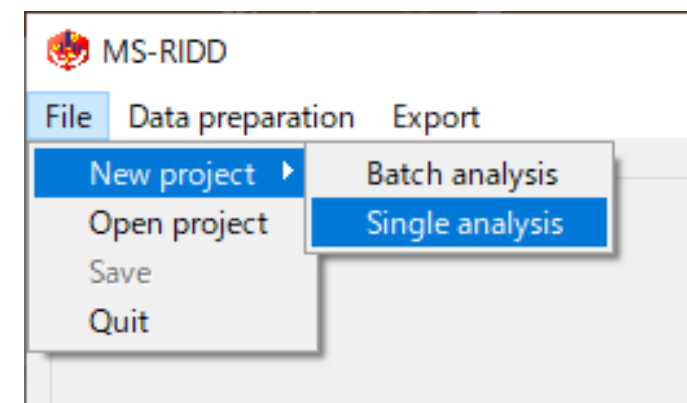
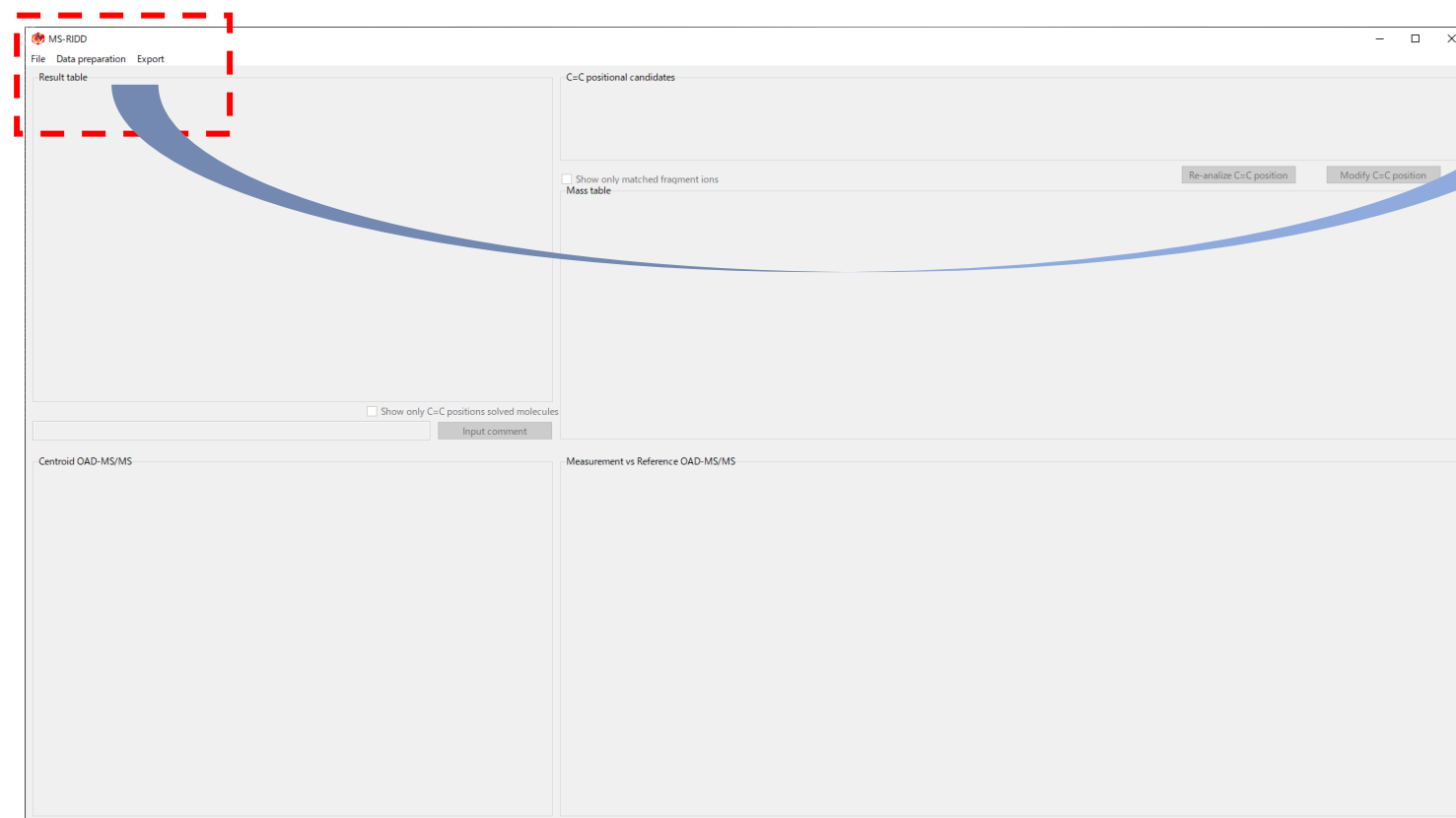
MS-RIDD tutorial

1-1. How to start OAD-MS/MS analysis



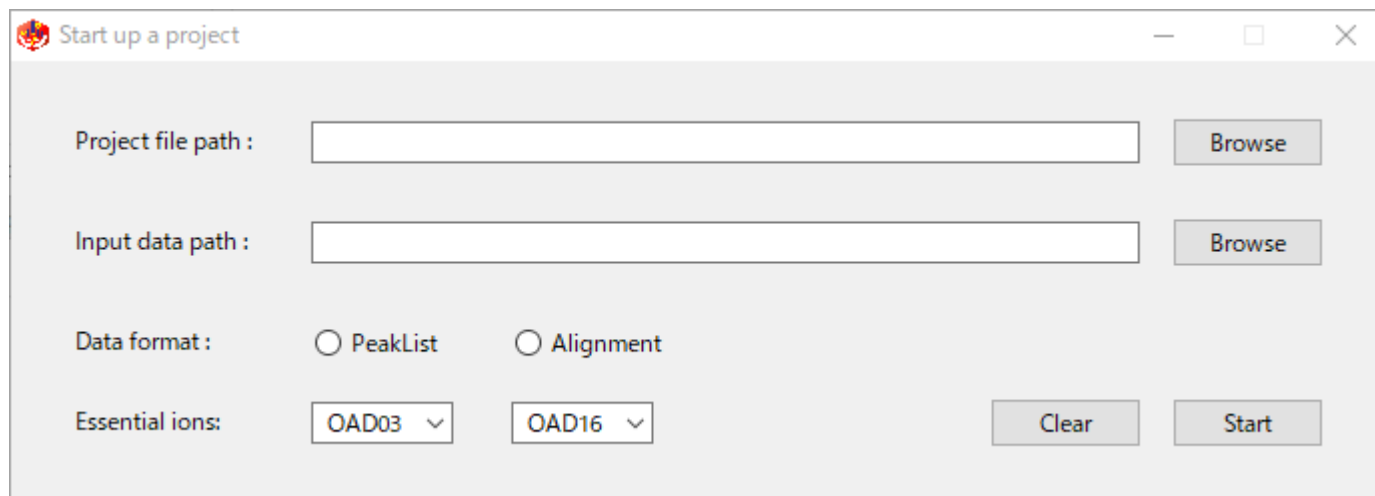
Open the folder of MS-RIDD, then
double click the .exe file (MS-RIDD.exe)

1-2a-1. How to start OAD-MS/MS analysis (New project / Single analysis)



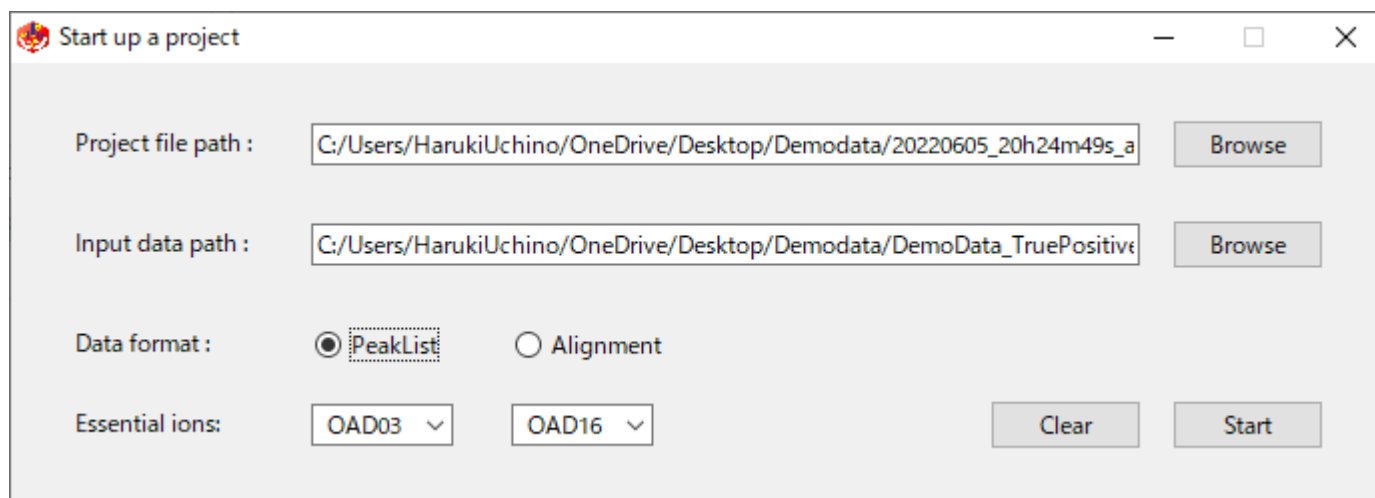
(For a single analysis)
After the GUI shown up, select the below
File > New project > Single analysis

1-2a-2. How to start OAD-MS/MS analysis (New project / Single analysis)



The 'Start up a project' dialog box is shown with the following fields and controls:

- Project file path :** An empty text box with a 'Browse' button to its right.
- Input data path :** An empty text box with a 'Browse' button to its right.
- Data format :** Two radio buttons: 'PeakList' (unselected) and 'Alignment' (unselected).
- Essential ions:** Two dropdown menus, both set to 'OAD03' and 'OAD16' respectively. To their right are 'Clear' and 'Start' buttons.



The 'Start up a project' dialog box is shown with the following fields and controls:

- Project file path :** The text box is populated with 'C:/Users/HarukiUchino/OneDrive/Desktop/Demodata/20220605_20h24m49s_a'. A 'Browse' button is to its right.
- Input data path :** The text box is populated with 'C:/Users/HarukiUchino/OneDrive/Desktop/Demodata/DemoData_TruePositive'. A 'Browse' button is to its right.
- Data format :** The 'PeakList' radio button is now selected, while 'Alignment' remains unselected.
- Essential ions:** The dropdown menus remain set to 'OAD03' and 'OAD16'. 'Clear' and 'Start' buttons are to the right.

Select the followings and click the start

Project file path:

select the folder where the analysis data generated by MS-RIDD locates

Input data path:

select the data (.txt) from MS-DIAL export containing OAD-MS/MS spectra

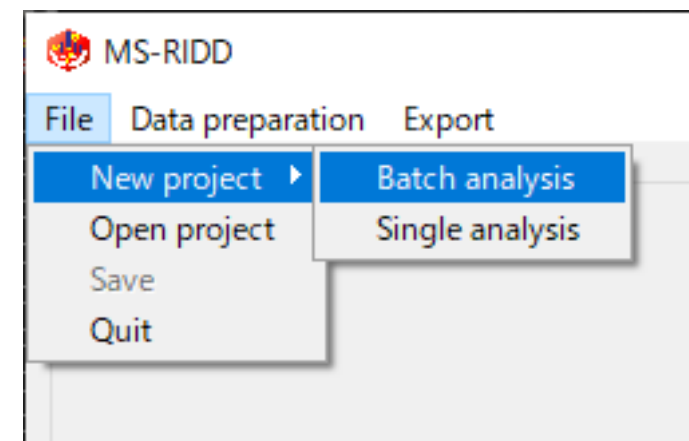
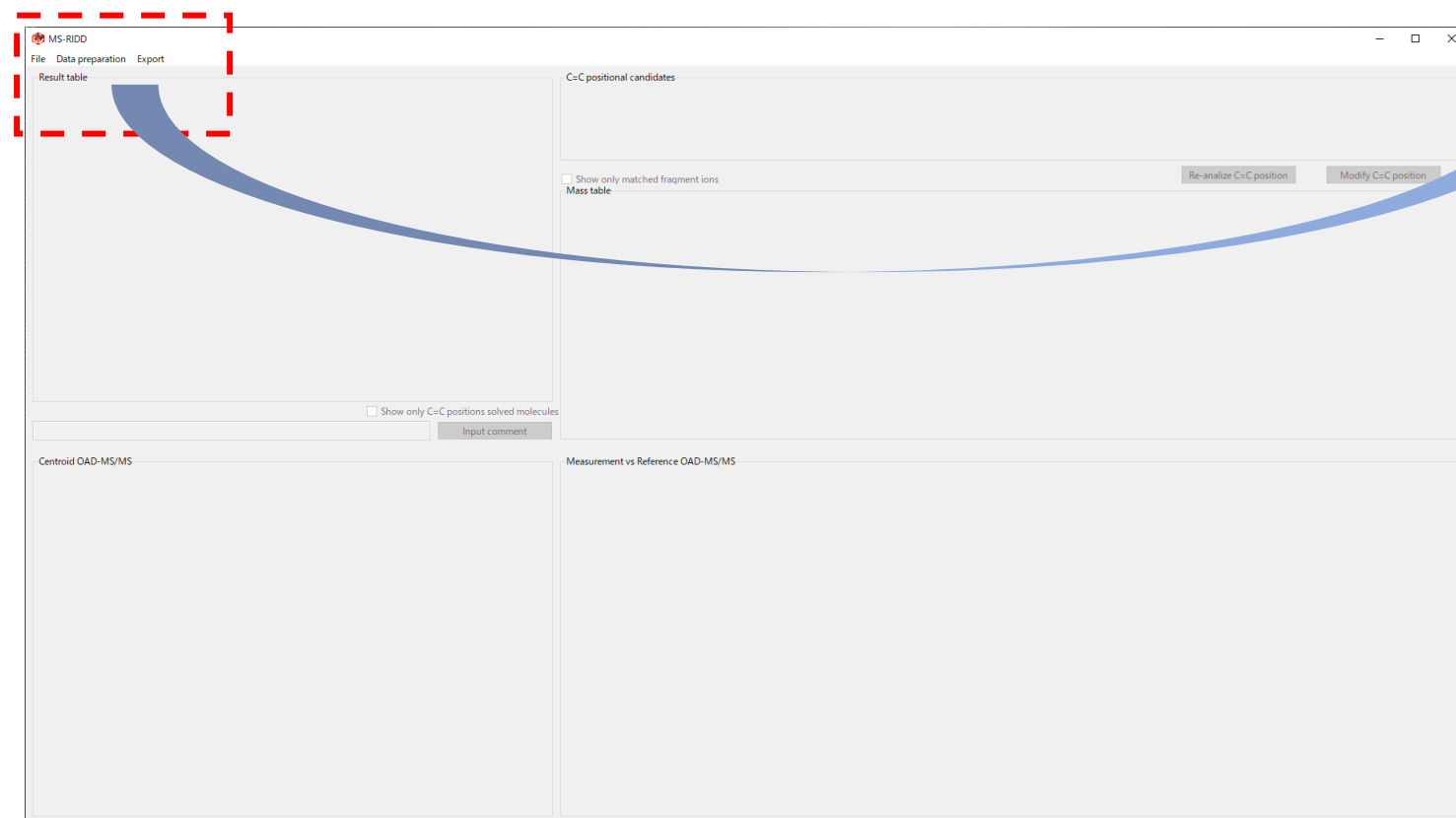
Data format:

select the data format in which MS-DIAL exported

Essential ions:

For the current data (doi.org/10.21203/rs.3.rs-727852/v1), select OAD03/OAD16

1-2b-1. How to start OAD-MS/MS analysis (New project / Batch analysis)

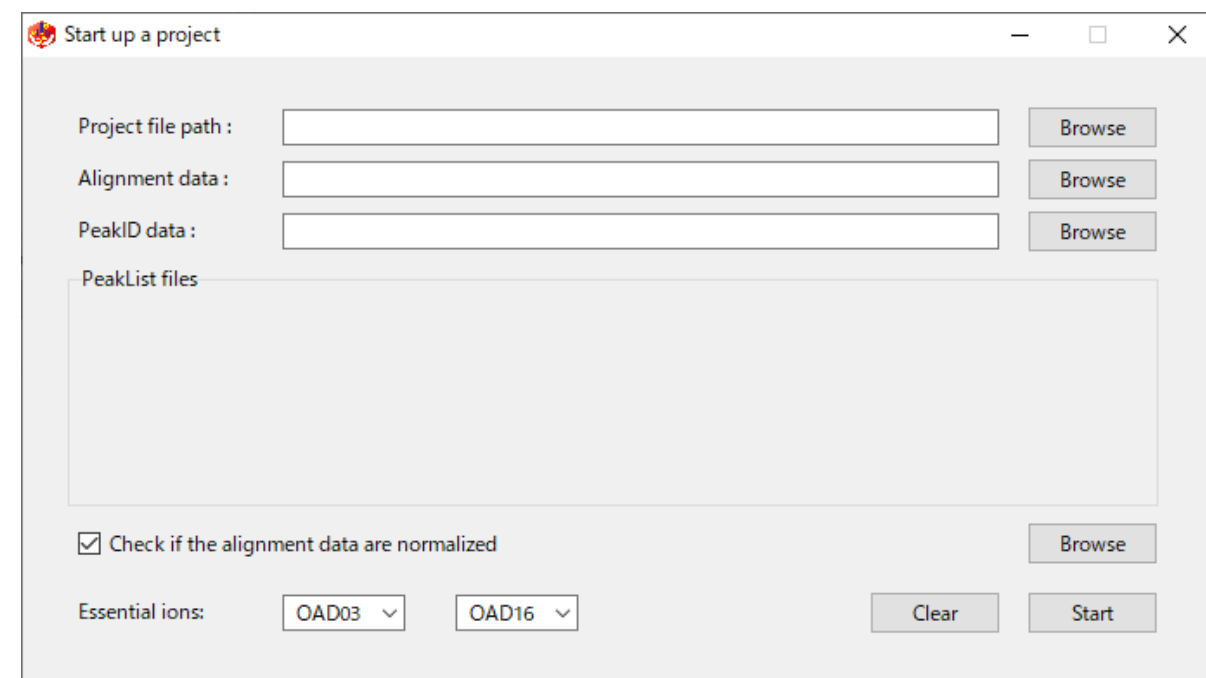


(For a batch analysis)

After the GUI shown up, select the below

File > New project > Batch analysis

1-2b-2. How to start OAD-MS/MS analysis (New project / Batch analysis)



Start up a project

Project file path : Browse

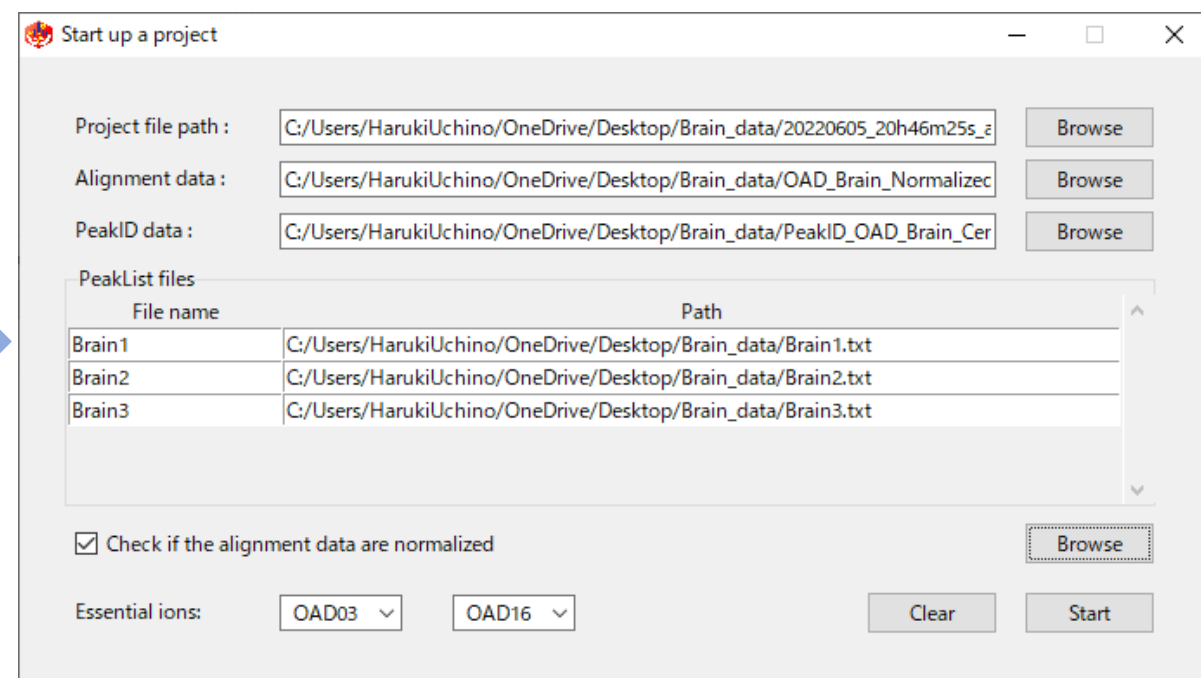
Alignment data : Browse

PeakID data : Browse

PeakList files

☒ Check if the alignment data are normalized Browse

Essential ions: OAD03 OAD16 Clear Start



Start up a project

Project file path : C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/20220605_20h46m25s_ε Browse

Alignment data : C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/OAD_Brain_Normalizedc Browse

PeakID data : C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/PeakID_OAD_Brain_Cer Browse

PeakList files

File name	Path
Brain1	C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/Brain1.txt
Brain2	C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/Brain2.txt
Brain3	C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/Brain3.txt

☒ Check if the alignment data are normalized Browse

Essential ions: OAD03 OAD16 Clear Start

Select the followings and click the start

Project file path:

select the folder where the analysis data generated by MS-RIDD locates

Alignment data path:

select the data (.txt) from MS-DIAL as alignment data format containing OAD-MS/MS spectra

PeakID data:

select the peak ID data from the same alignment data

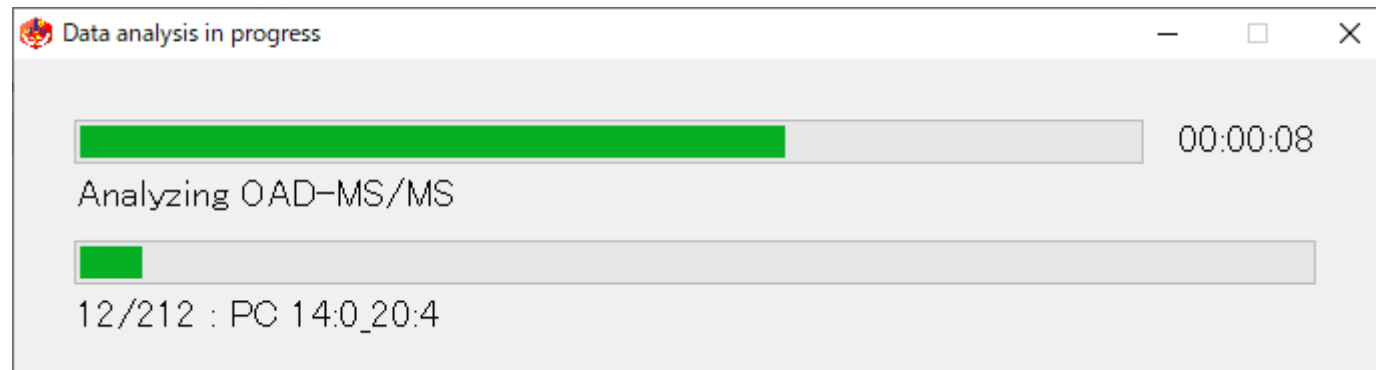
PeakList files:

select each sample data of the alignment data as PeakList format of MS-DIAL export

Essential ions:

For the current data (doi.org/10.21203/rs.3.rs-727852/v1), select OAD03/OAD16

1-3. How to start OAD-MS/MS analysis (Processing)



Please wait until the processing finish

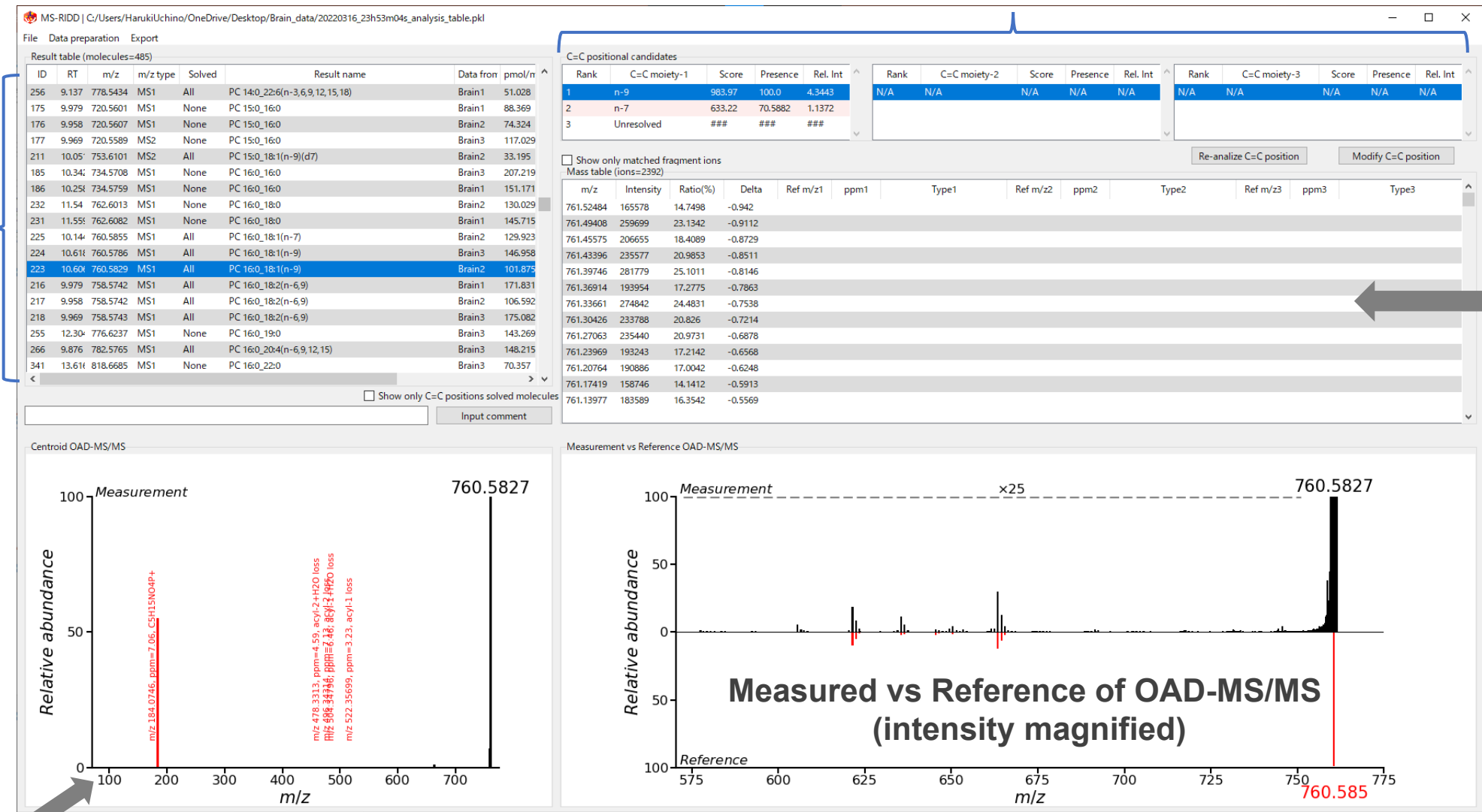
*** Error will occur if the data format is not correct**

2-1. How to check the automatic result of OAD-MS/MS analysis

Graphical use interface of MS-RIDD

Candidates of C=C positions for each unsaturated moiety

Metabolite
table



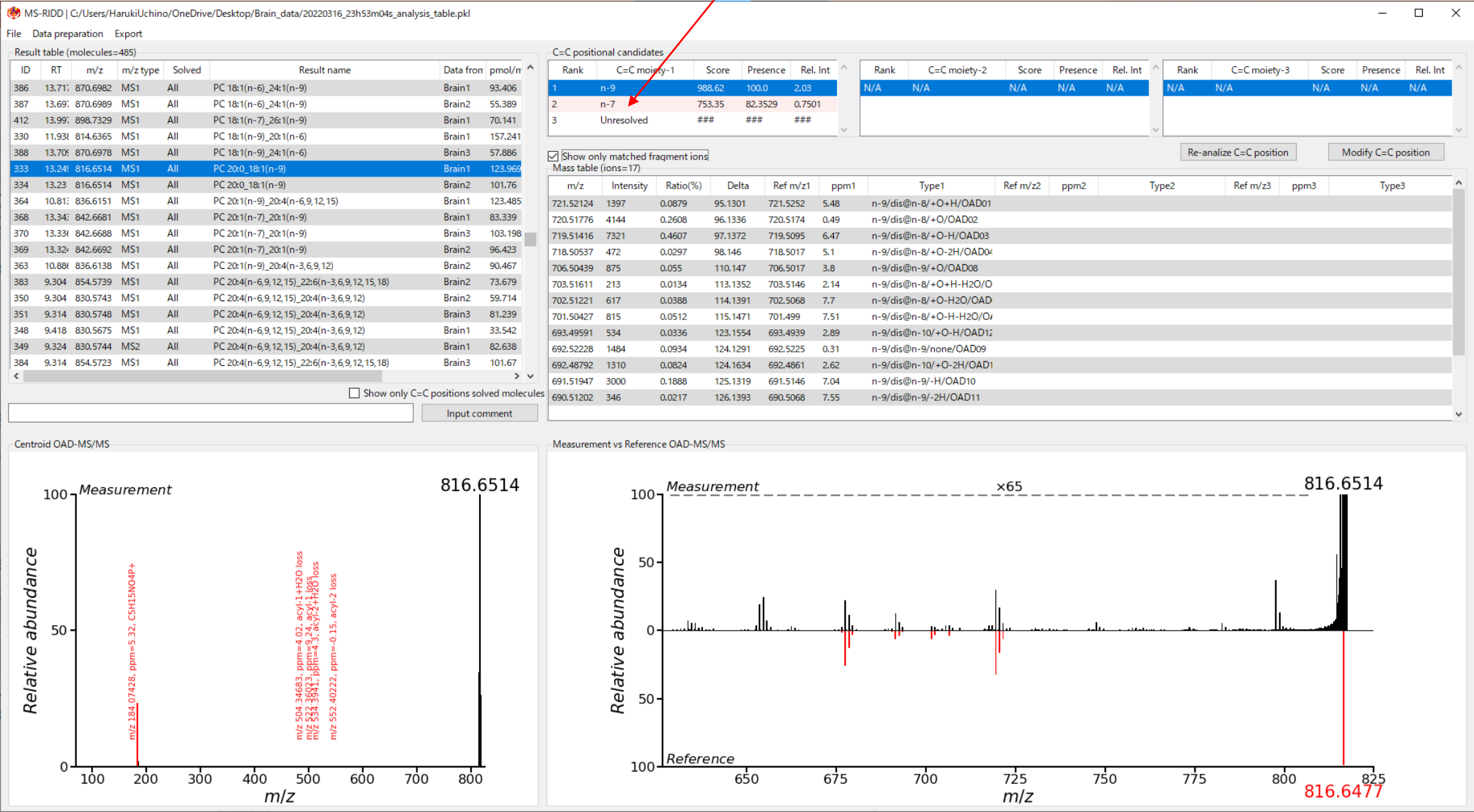
Mass table

Measured of OAD-MS/MS

* If the fragment ions for the annotation of acyl-chain isomer levels are detected (based on the CID), red colored annotation labels will show up

2-1. How to check the automatic result of OAD-MS/MS analysis (Modify the annotation of C=C position)

Click the other C=C candidates for checking the spectrum matching



2-1. How to check the automatic result of OAD-MS/MS analysis (Modify the annotation of C=C position)

If you want to change the annotation as current C=C position,
Click “Modify C=C position”

MS-RIDD | C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/20220316_23h53m04s_analysis_table.pkl

File Data preparation Export

Result table (molecules=485)

ID	RT	m/z	m/z type	Solved	Result name	Data from	pmol/n
386	13.71	870.6982	MS1	All	PC 18:1(n-6)_24:1(n-9)	Brain1	93.406
387	13.69	870.6989	MS1	All	PC 18:1(n-6)_24:1(n-9)	Brain2	55.389
412	13.99	898.7329	MS1	All	PC 18:1(n-7)_26:1(n-9)	Brain1	70.141
330	11.93	814.6365	MS1	All	PC 18:1(n-9)_20:1(n-6)	Brain1	157.241
388	13.70	870.6978	MS1	All	PC 18:1(n-9)_24:1(n-6)	Brain3	57.886
333	13.24	816.6514	MS1	All	PC 20:0_18:1(n-9)	Brain1	123.969
334	13.23	816.6514	MS1	All	PC 20:0_18:1(n-9)	Brain2	101.76
364	10.81	836.6151	MS1	All	PC 20:1(n-9)_20:4(n-6,9,12,15)	Brain1	123.485
368	13.34	842.6681	MS1	All	PC 20:1(n-7)_20:1(n-9)	Brain1	83.339
370	13.33	842.6688	MS1	All	PC 20:1(n-7)_20:1(n-9)	Brain3	103.198
369	13.32	842.6692	MS1	All	PC 20:1(n-7)_20:1(n-9)	Brain2	96.423
363	10.88	836.6138	MS1	All	PC 20:1(n-9)_20:4(n-3,6,9,12)	Brain2	90.467
383	9.304	854.5739	MS1	All	PC 20:4(n-6,9,12,15)_22:6(n-3,6,9,12,15,18)	Brain2	73.679
350	9.304	830.5743	MS1	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain2	59.714
351	9.314	830.5748	MS1	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain3	81.239
348	9.418	830.5675	MS1	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain1	33.542
349	9.324	830.5744	MS2	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain1	82.638
384	9.314	854.5723	MS1	All	PC 20:4(n-6,9,12,15)_22:6(n-3,6,9,12,15,18)	Brain3	101.67

☐ Show only C=C positions solved molecules

☒ Show only matched fragment ions

Mass table (ions=14)

m/z	Intensity	Ratio(%)	Delta	Ref m/z1	ppm1	Type1	Ref m/z2	ppm2	Type2	Ref m/z3	ppm3	Type3
749.55316	265	0.0166	67.0982	749.5565	4.45	n-7/dis@n-6/+O+H/OAD01						
748.54559	553	0.0348	68.1058	748.5487	4.15	n-7/dis@n-6/+O/OAD02						
747.547	1455	0.0915	69.1043	747.5408	8.29	n-7/dis@n-6/+O-H/OAD03						
746.54504	205	0.0129	70.1063	746.533	16.12	n-7/dis@n-6/+O-2H/OAD04						
734.53857	278	0.0174	82.1128	734.533	7.58	n-7/dis@n-7/+O/OAD08						
731.54852	107	0.0067	85.1028	731.5459	3.58	n-7/dis@n-6/+O+H-H2O/O						
730.5401	492	0.0309	86.1112	730.5381	2.73	n-7/dis@n-6/+O-H2O/OAD						
729.52661	156	0.0098	87.1247	729.5303	5.05	n-7/dis@n-6/+O-H-H2O/O						
721.52124	1397	0.0879	95.1301	721.5252	5.48	n-7/dis@n-8/+O-H/OAD12						
720.51776	4144	0.2608	96.1336	720.5174	0.49	n-7/dis@n-8/+O-2H/OAD13						
718.54218	866	0.0545	98.1092	718.5381	5.67	n-7/dis@n-7/-2H/OAD11						
706.53619	791	0.0497	110.1152	706.5381	2.7	n-7/dis@n-8/none/OAD15						
705.53345	912	0.0574	111.1179	705.5303	4.46	n-7/dis@n-8/-H/OAD16						

Re-analyze C=C position

Modify C=C position

Input comment

Centroid OAD-MS/MS

Measurement vs Reference OAD-MS/MS

2-1. How to check the automatic result of OAD-MS/MS analysis (Modify the annotation of C=C position)

Then, the annotation name on the metabolite table will change
(annotation information on mass table and reference OAD-MS/MS are
Changed as well.)

MS-RIDD | C:/Users/HarukiUchino/OneDrive/Desktop/Brain_data/20220316_23h53m04s_analysis_table.pkl

File Data preparation Export

Result table (molecules=485)

ID	RT	m/z	m/z type	Solved	Result name	Data from	pmol/n
386	13.71	870.6982	MS1	All	PC 18:1(n-6)_24:1(n-9)	Brain1	93.406
387	13.69	870.6989	MS1	All	PC 18:1(n-6)_24:1(n-9)	Brain2	55.389
412	13.99	898.7329	MS1	All	PC 18:1(n-7)_26:1(n-9)	Brain1	70.141
330	11.93	814.6365	MS1	All	PC 18:1(n-9)_20:1(n-6)	Brain1	157.241
388	13.70	870.6978	MS1	All	PC 18:1(n-9)_24:1(n-6)	Brain3	57.886
333	13.24	816.6514	MS1	All	PC 20:0_18:1(n-7)	Brain1	123.969
334	13.23	816.6514	MS1	All	PC 20:0_18:1(n-9)	Brain2	101.76
364	10.81	836.6151	MS1	All	PC 20:1(n-9)_20:4(n-6,9,12,15)	Brain1	123.485
368	13.34	842.6681	MS1	All	PC 20:1(n-7)_20:1(n-9)	Brain1	83.339
370	13.33	842.6688	MS1	All	PC 20:1(n-7)_20:1(n-9)	Brain3	103.198
369	13.32	842.6692	MS1	All	PC 20:1(n-7)_20:1(n-9)	Brain2	96.423
363	10.88	836.6138	MS1	All	PC 20:1(n-9)_20:4(n-3,6,9,12)	Brain2	90.467
383	9.304	854.5739	MS1	All	PC 20:4(n-6,9,12,15)_22:6(n-3,6,9,12,15,18)	Brain2	73.679
350	9.304	830.5743	MS1	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain2	59.714
351	9.314	830.5748	MS1	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain3	81.239
348	9.418	830.5675	MS1	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain1	33.542
349	9.324	830.5744	MS2	All	PC 20:4(n-6,9,12,15)_20:4(n-3,6,9,12)	Brain1	82.638
384	9.314	854.5723	MS1	All	PC 20:4(n-6,9,12,15)_22:6(n-3,6,9,12,15,18)	Brain3	101.67

☐ Show only C=C positions solved molecules

Input comment

C=C positional candidates

Rank	C=C moiety-1	Score	Presence	Rel. Int
1	n-7	753.35	82.3529	0.7501
2	n-9	988.62	100.0	2.03
3	Unresolved	###	###	###

Rank	C=C moiety-2	Score	Presence	Rel. Int
N/A	N/A	N/A	N/A	N/A

Rank	C=C moiety-3	Score	Presence	Rel. Int
N/A	N/A	N/A	N/A	N/A

Re-analyze C=C position

Modify C=C position

☒ Show only matched fragment ions

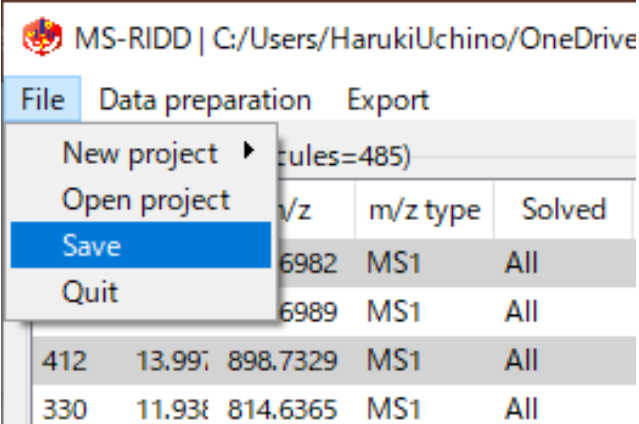
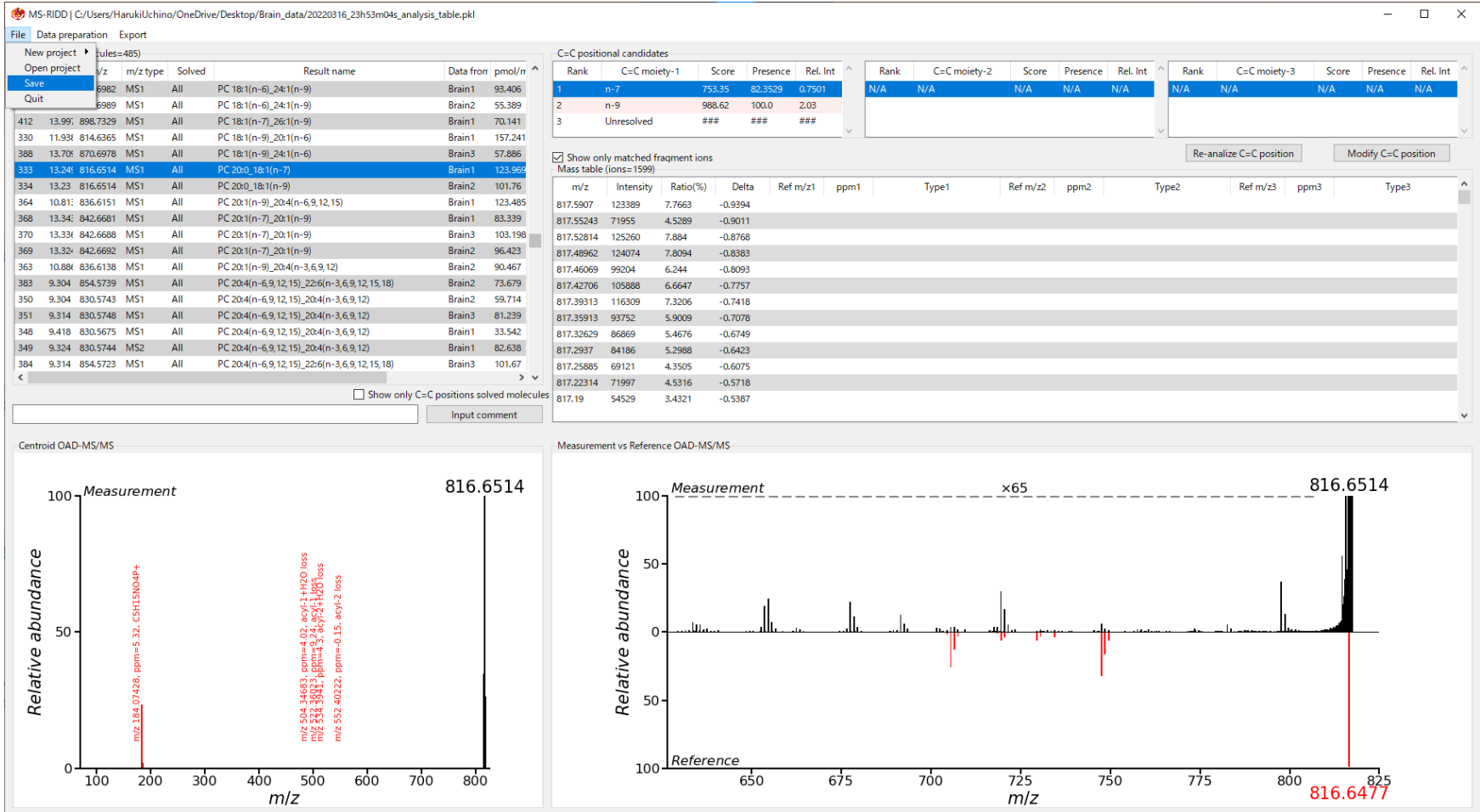
Mass table (ions=1599)

m/z	Intensity	Ratio(%)	Delta	Ref m/z1	ppm1	Type1	Ref m/z2	ppm2	Type2	Ref m/z3	ppm3	Type3
817.5907	123389	7.7663	-0.9394									
817.55243	71955	4.5289	-0.9011									
817.52814	125260	7.884	-0.8768									
817.48962	124074	7.8094	-0.8383									
817.46069	99204	6.244	-0.8093									
817.42706	105888	6.6647	-0.7757									
817.39313	116309	7.3206	-0.7418									
817.35913	93752	5.9009	-0.7078									
817.32629	86869	5.4676	-0.6749									
817.2937	84186	5.2988	-0.6423									
817.25885	69121	4.3505	-0.6075									
817.22314	71997	4.5316	-0.5718									
817.19	54529	3.4321	-0.5387									

Centroid OAD-MS/MS

Measurement vs Reference OAD-MS/MS

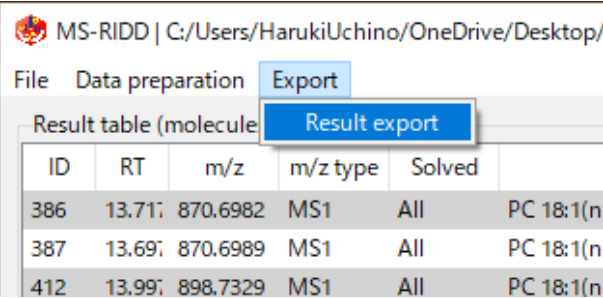
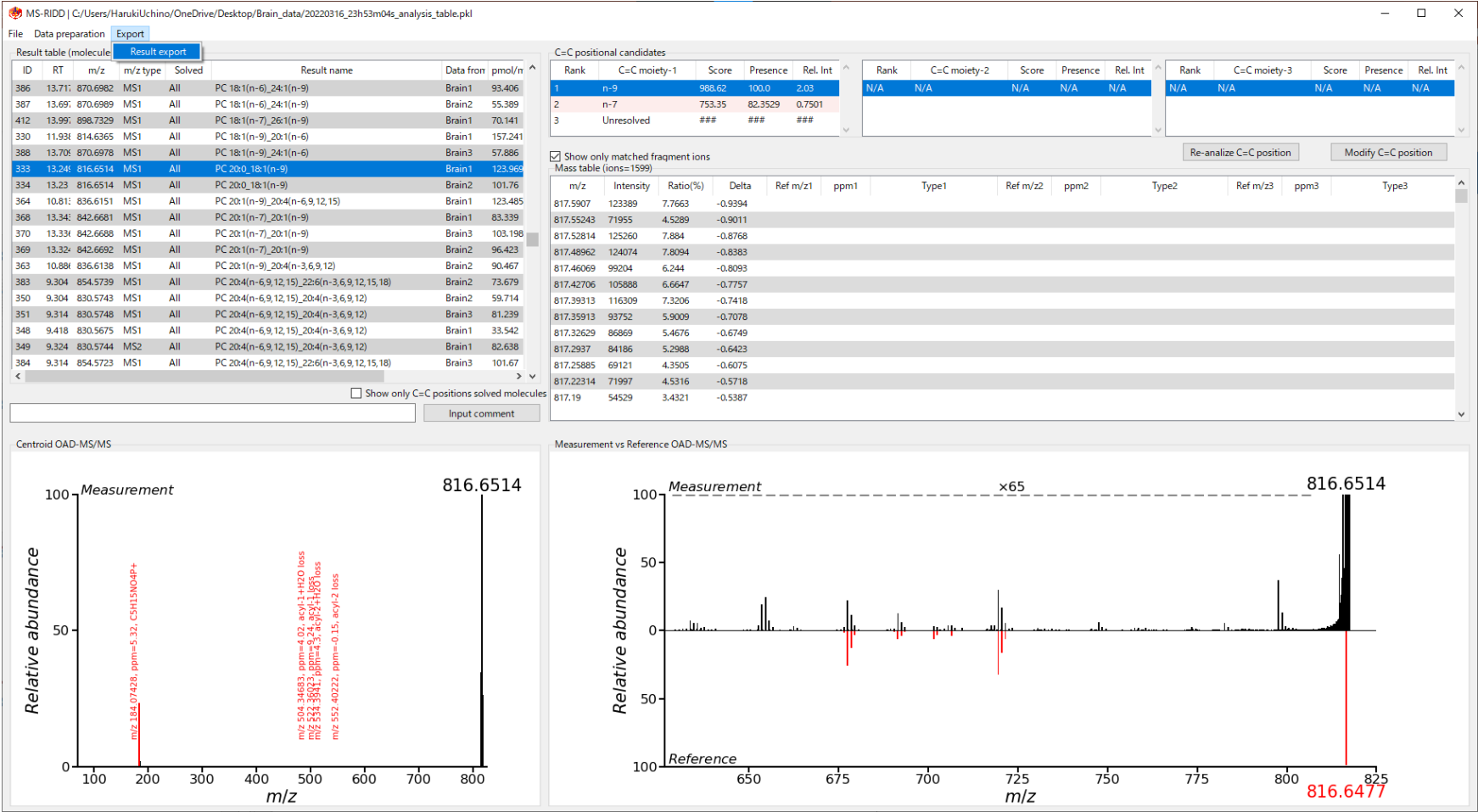
3-1. How to save the analysis data



Select: File > Save

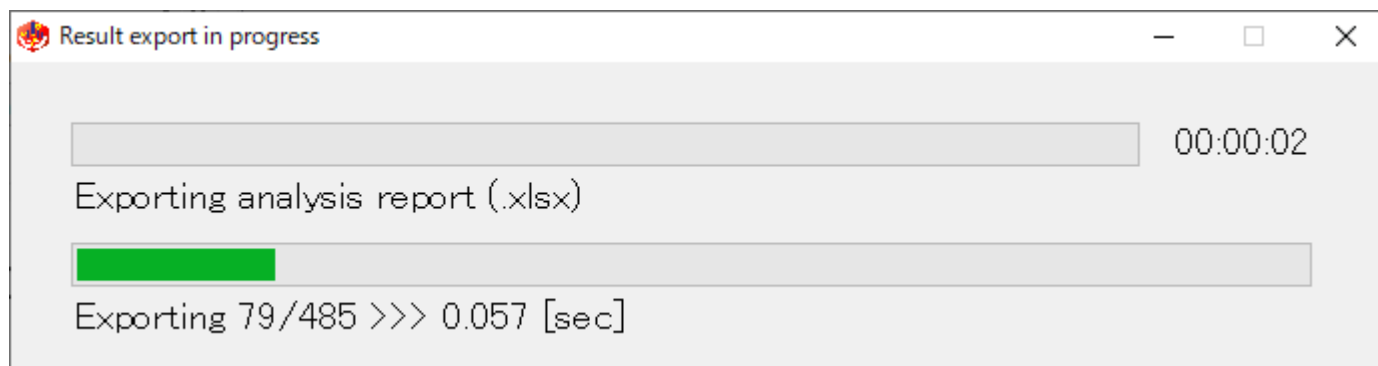
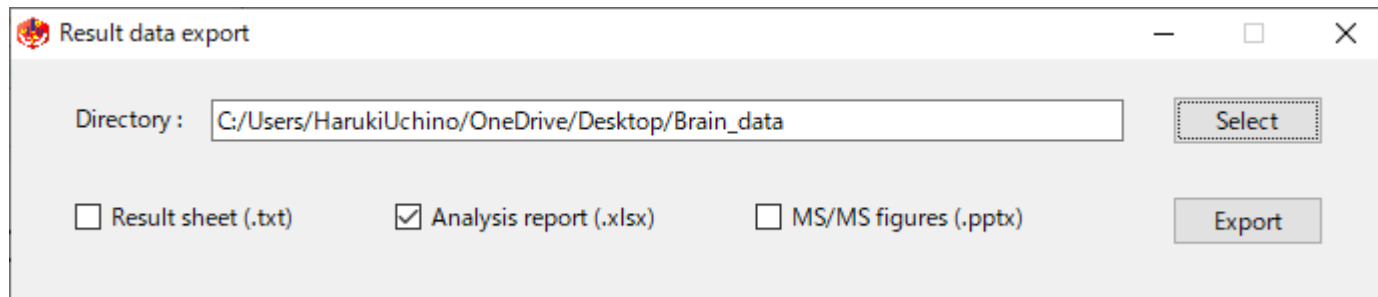
* Saving is needed for the information which user manually modified and/or added.

4-1. How to export the analysis data



Select: Export > Result expot

4-1. How to export the analysis data



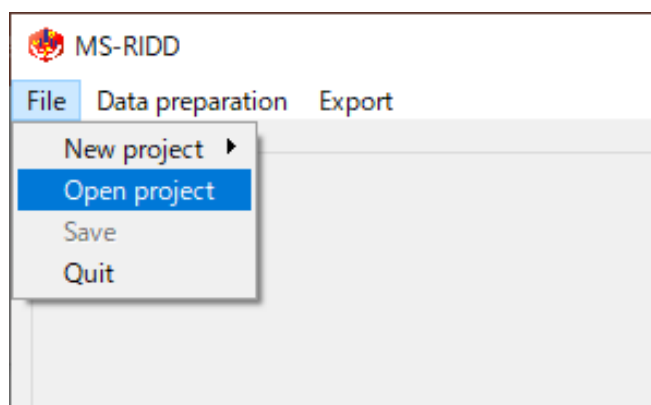
Select the followings and click “Export”

Directory:

select the folder where the selected result data generated by MS-RIDD locates

* For default setting, “Analysis report” is selected. Exporting MS/MS figures (.pptx) takes several minutes.

5-1. How to open the previous analysis data



Select “Open project”,
then select the MS-RIDD data (.pkl).

* The following data are also
required to be in the same folder
as the MS-RIDD data (.pkl).

- “ *_cid_result.pickle”
- “ *_extracted_msms.pickle”
- “ *_graph_info.pickle”
- “ *_oad_result.pickle”
- “ *_structure_info.pickle”

