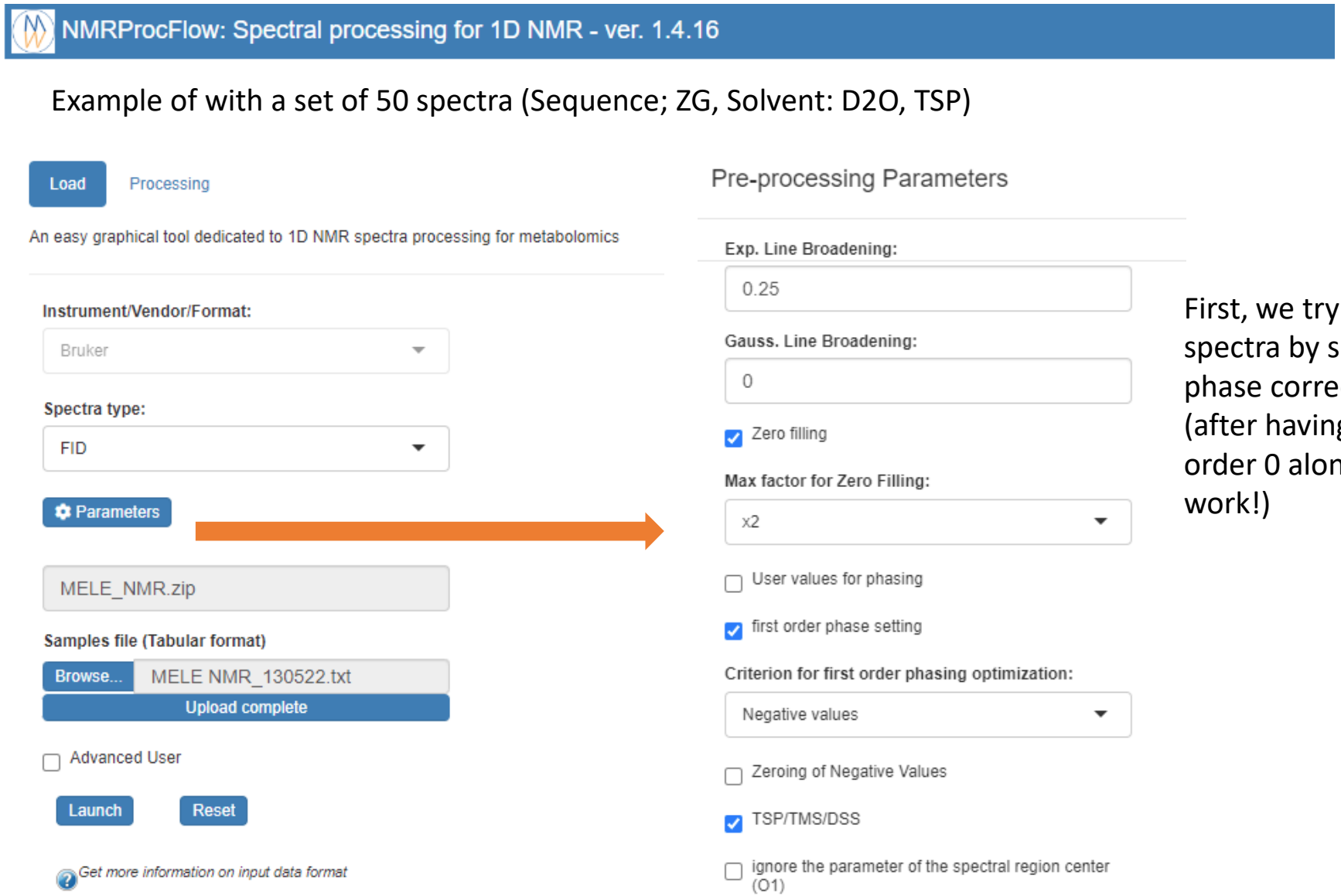
NMRProcFlow

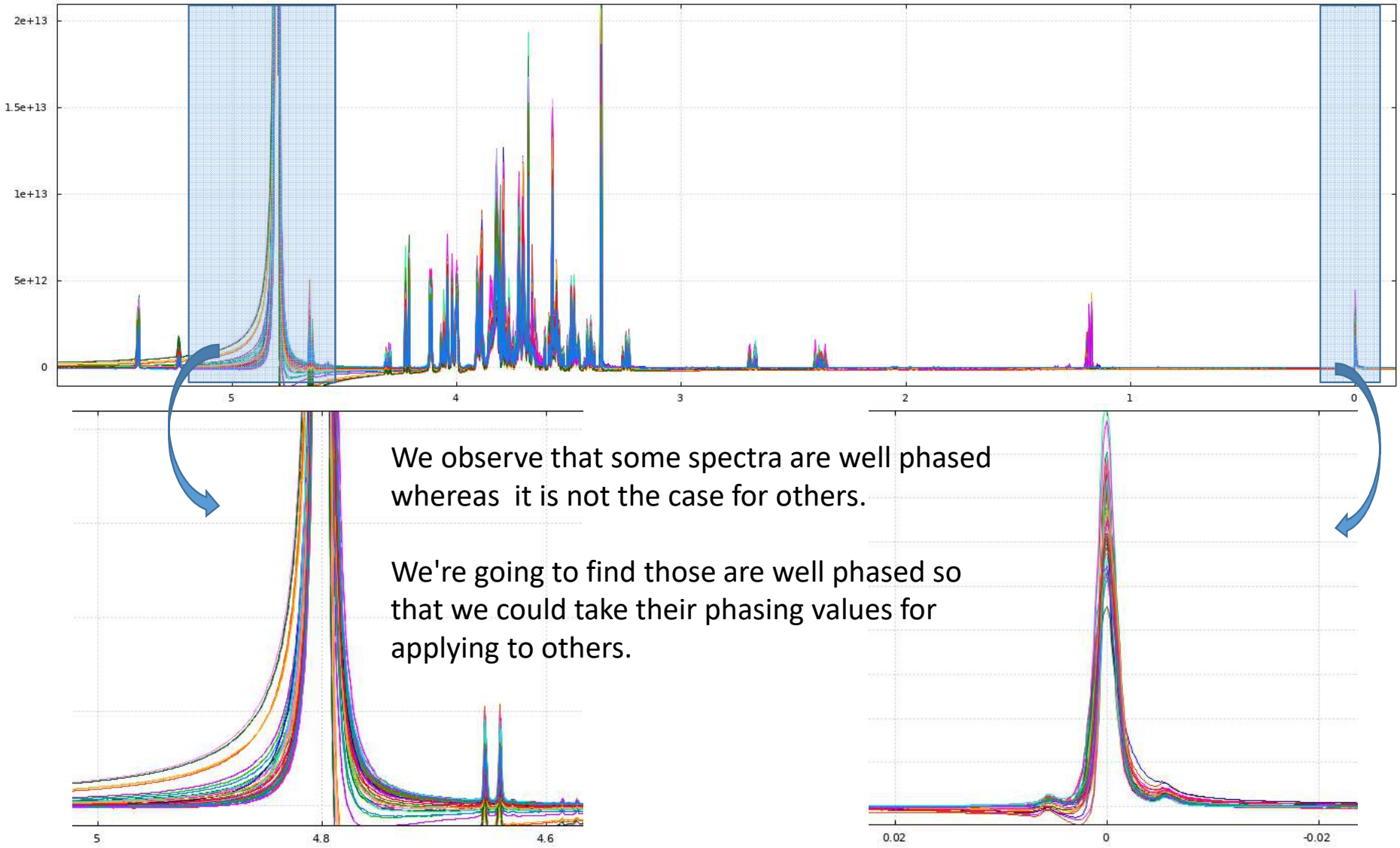
How to properly phase a whole set of spectra when NMRProcFlow fails on some spectra

Daniel Jacob

INRAE

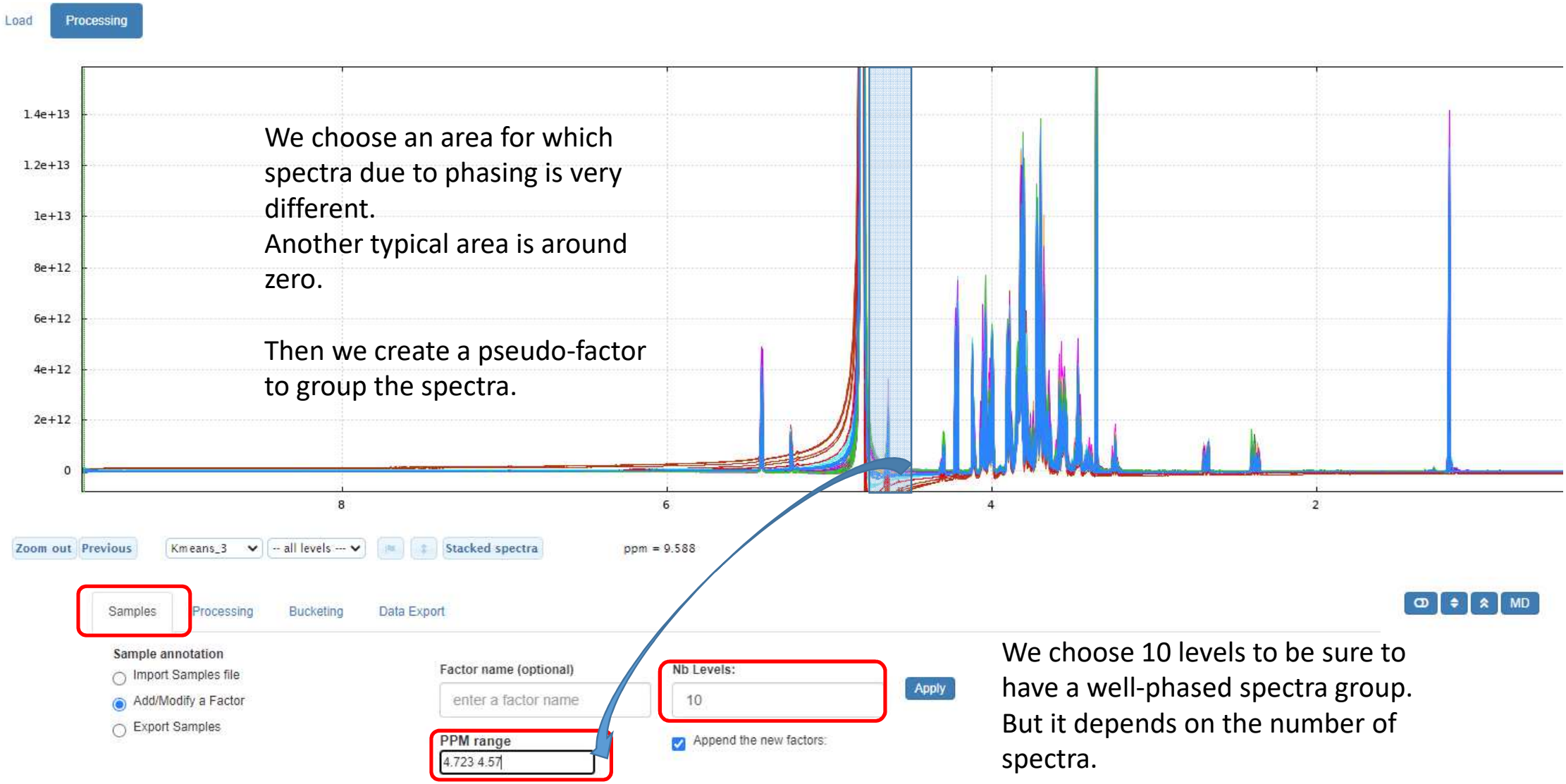


First, we try to phase all the spectra by setting the phase correction to order 1 (after having noticed that order 0 alone does not work!)

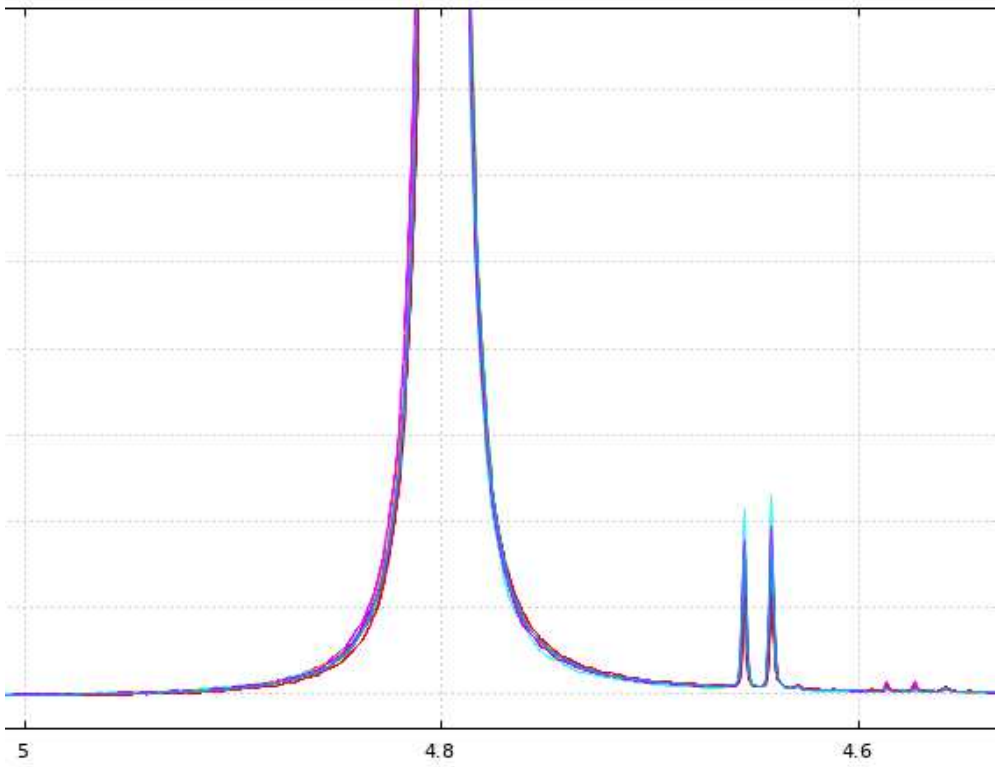


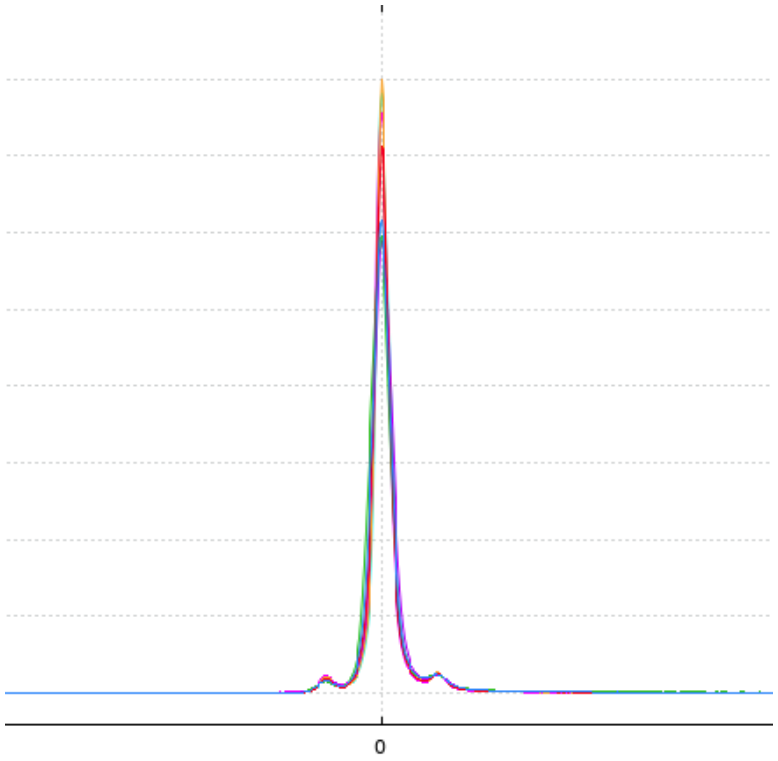
We observe that some spectra are well phased whereas it is not the case for others.

We're going to find those are well phased so that we could take their phasing values for applying to others.



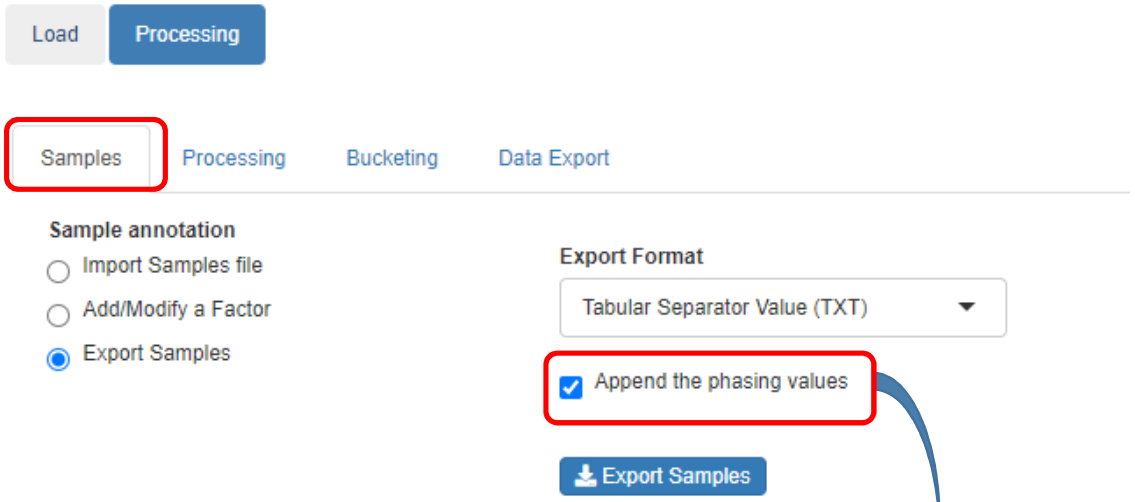
We choose 10 levels to be sure to have a well-phased spectra group. But it depends on the number of spectra.





Pseudo-factor

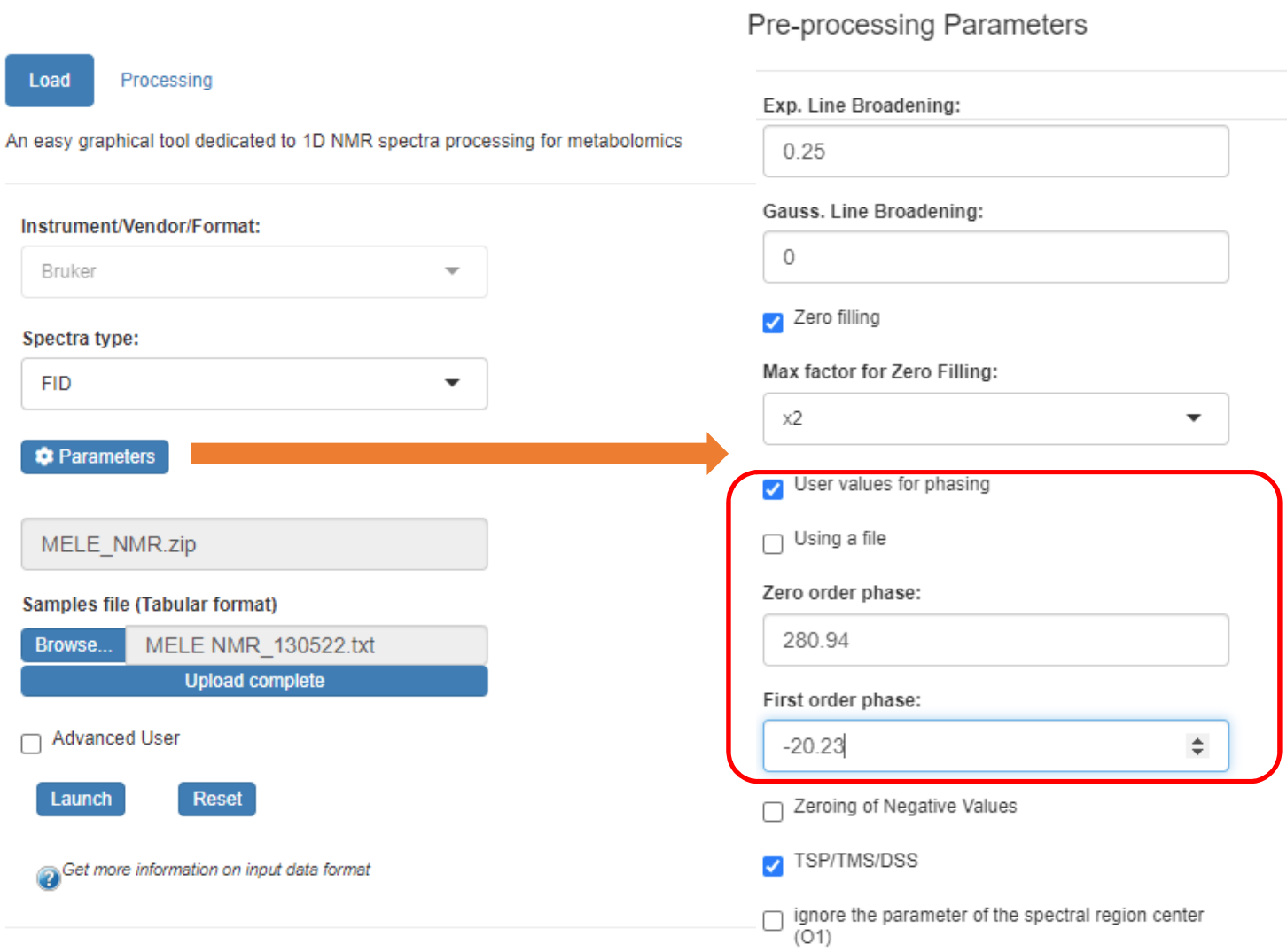
In our case, the L10 level spectra of this pseudo-factor seem to be perfectly well phased.



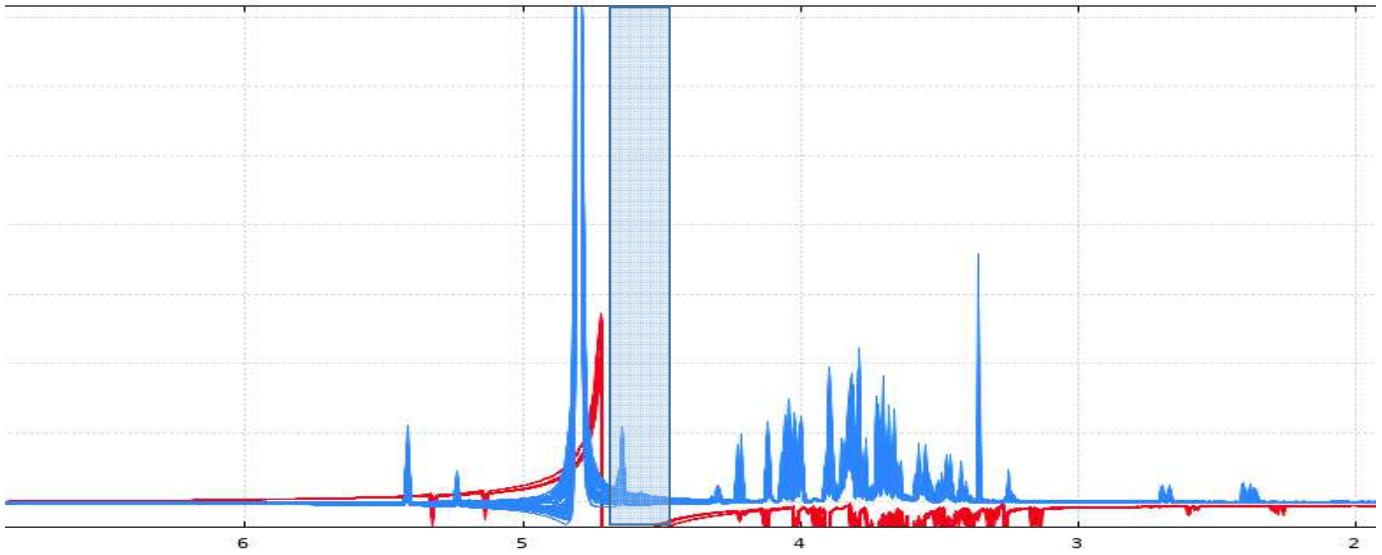
Export the sample file having for each sample the phasing values and the corresponding level for the pseudo factor.

	A	B	C	D	E	F	G	H	I
1	Spectrum	Samplecode	EXPNO	PROCNO	PHC0	PHC1	Kmeans_3		
2	M2_II	M2_II	1	1	280.378879	-20.41467211	L10	280.937389	-20.2287251
3	M4_I	M4_I	1	1	279.716102	-22.02765457	L10		
4	M8_III	M8_III	1	1	281.342564	-20.79354173	L10		
5	M10_III	M10_III	1	1	282.798548	-18.76771326	L10		
6	M15_I	M15_I	1	1	280.259181	-20.26354568	L10		
7	M15_III	M15_III	1	1	281.75918	-18.57970226	L10		
8	M17_III	M17_III	1	1	280.307272	-20.7542462	L10		
9	M9_III	M9_III	1	1	279.490726	-19.42482321	L09		
10	M12_I	M12_I	1	1	284.636796	-25.87253245	L09		
11	M1_II	M1_II	1	1	271.205202	10.47080716	L08		
12	M4_III	M4_III	1	1	273.951678	9.23248355	L08		
13	M6_II	M6_II	1	1	272.265278	10.53185219	L08		
14	M13_I	M13_I	1	1	99.3378604	14.54810977	L08		
15	M3_II	M3_II	1	1	283.724099	-18.5556763	L07		
16	M7_II	M7_II	1	1	279.701882	-8.673038806	L07		

Then we calculate the average of the phage values for the group chosen as the best phased.



We reload the set of spectra by specifying the previously calculated phasing values.



In the same way as before, a pseudo-factor is created, but this time to identify the group of badly phased spectra.

Zoom out | Previous | Kmeans_3 | -- all levels --- | Stacked spectra | ppm = 9.305

Samples | Processing | Bucketing | Data Export

Sample annotation

- ○ Import Samples file
- ◉ Add/Modify a Factor
- ○ Export Samples

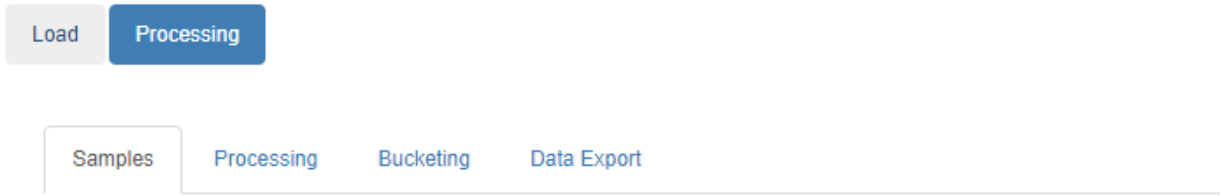
Factor name (optional): enter a factor name

Nb Levels: 2

Apply

PPM range: 4.763 4.384

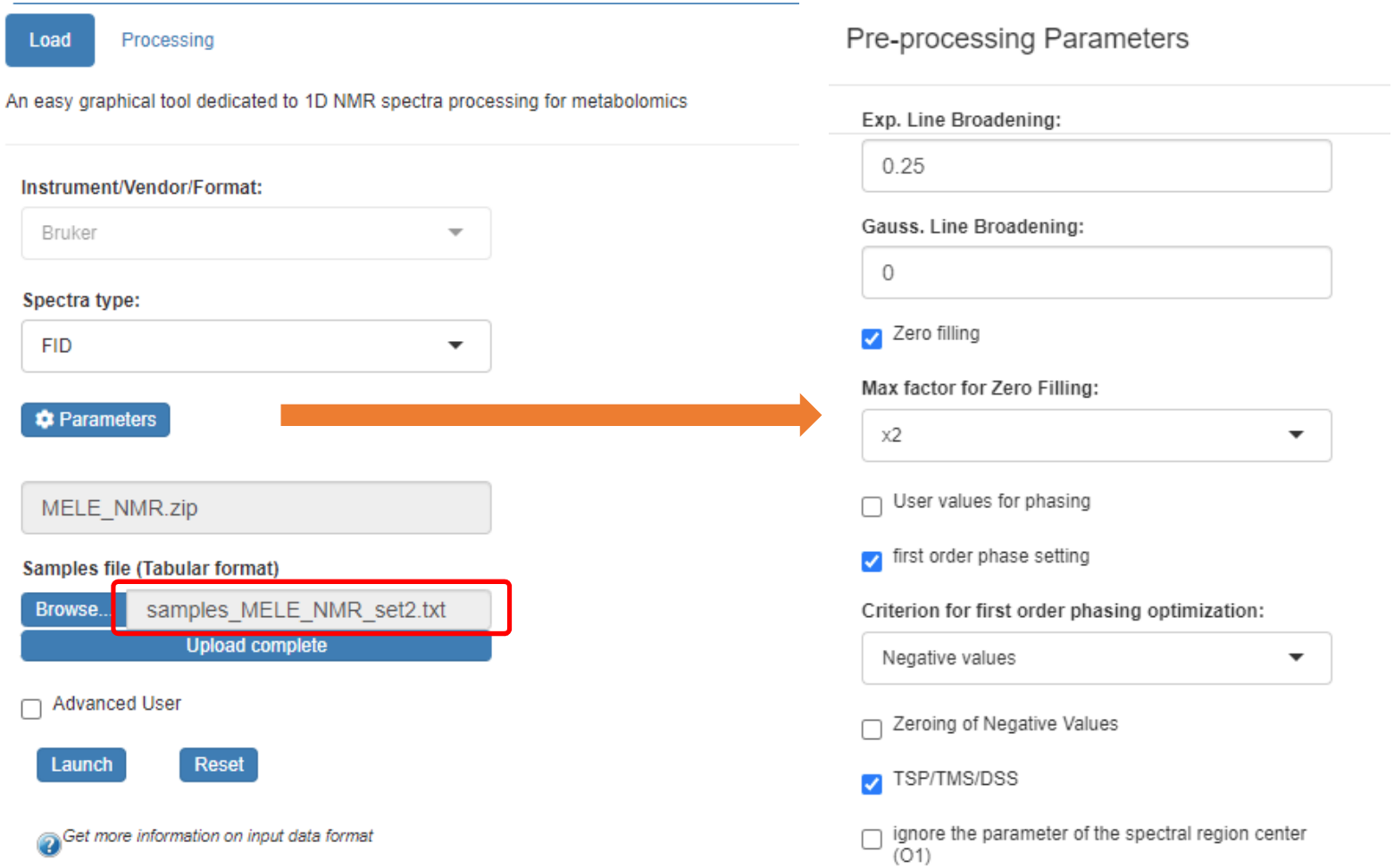
☑ Append the new factors:



	A	B	C	D	E	F	G
1	Spectrum	Samplecode	Expno	Procno	varieties	Origins	Kmeans_3
2	M9_I	M9_I	1	1	Calve	Toscana_Regior	L02
3	M10_I	M10_I	1	1	Cipolla_di_C	Toscana_Regior	L02
4	M11_I	M11_I	1	1	Cipollana	Toscana_Regior	L02
5	M13_I	M13_I	1	1	Nesta	Toscana_Regior	L02
6	M17_I	M17_I	1	1	Sassola	Toscana_Regior	L02
7							

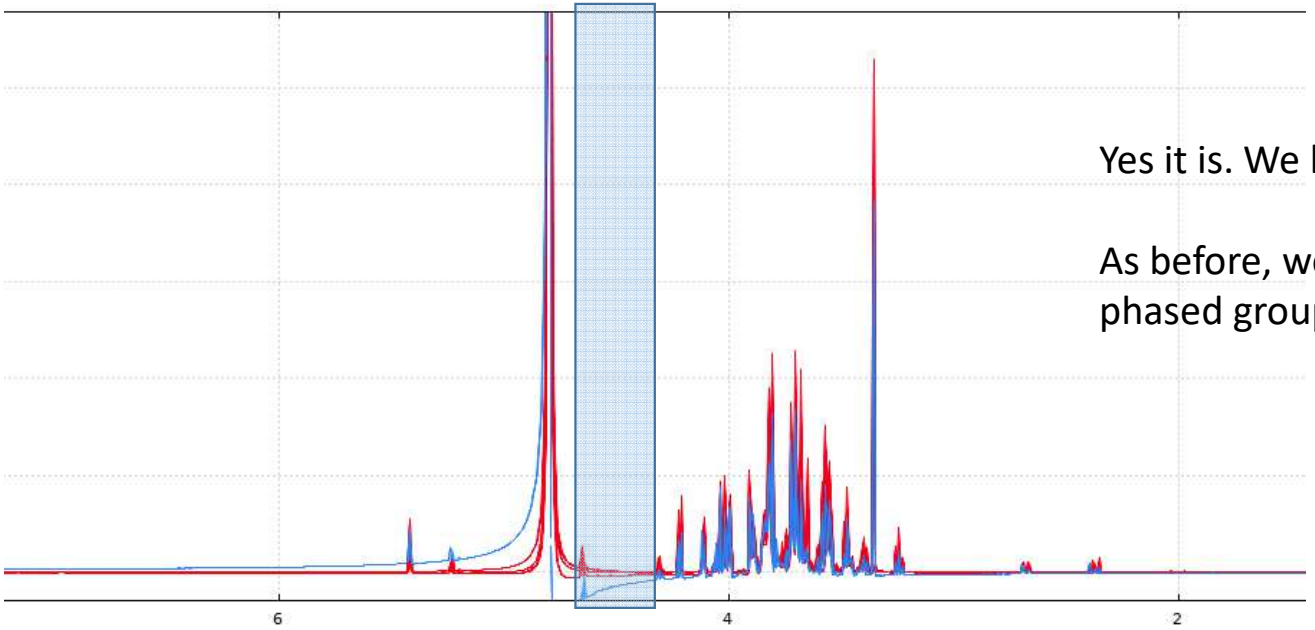
Only the level corresponding to the group of badly phased spectra is kept

samples_MELE_NMR_set2.txt



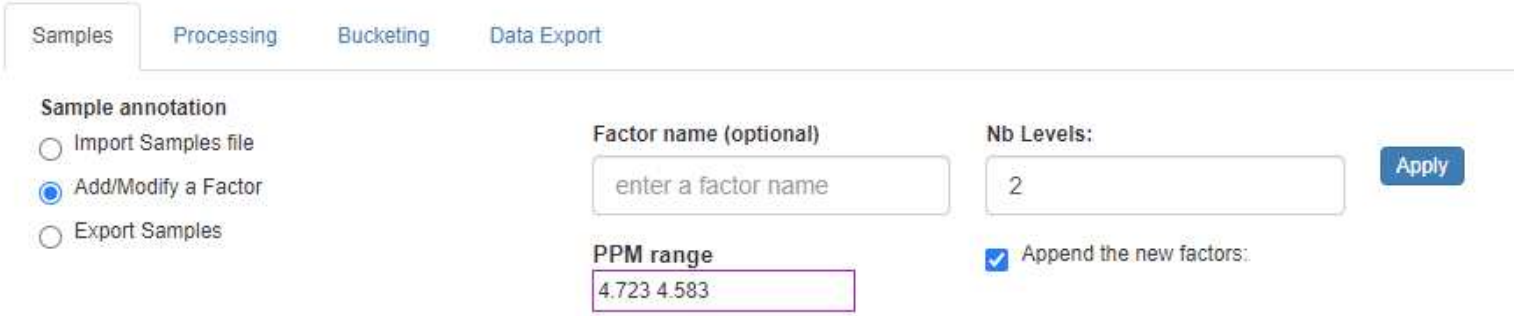
The set of spectra is reloaded, but only the previous poorly phased group is kept.

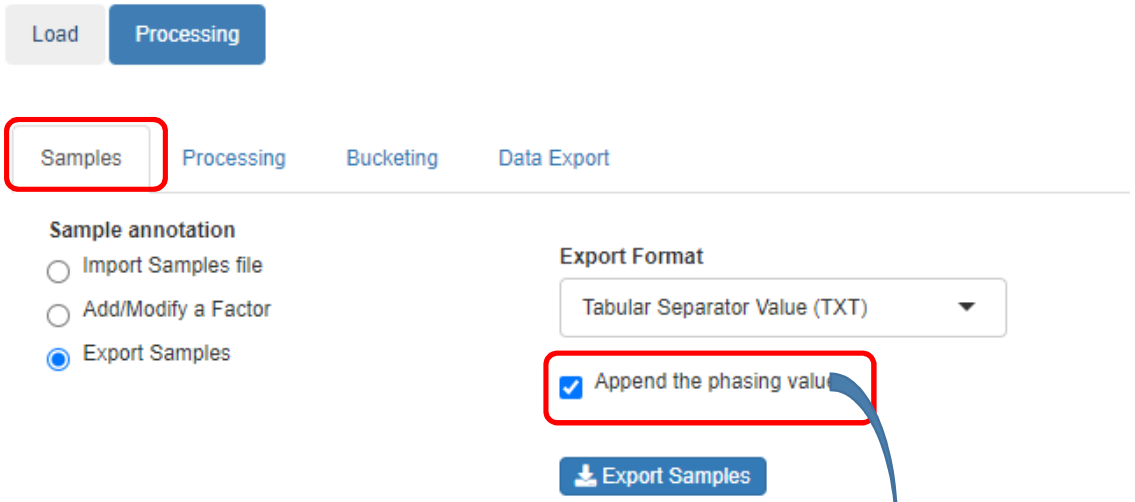
Here we want to know if among this group of spectra, there are some that would be correctly phased and from which we could obtain the phasing values.



Yes it is. We have a subgroup that is well phased.

As before, we create a pseudo-factor to select the well phased group.





Export the sample file having for each sample the phasing values and the corresponding level for the pseudo factor.

	A	B	C	D	E	F	G	H	I
1	Spectrum	Samplecode	EXPNO	PROCNO	PHC0	PHC1	Kmeans_3		
2	M13_I	M13_I	1	1	99.3378604	14.5481098	L01		
3	M10_I	M10_I	1	1	110.989968	-18.5321381	L02	109.109232	-17.447461
4	M11_I	M11_I	1	1	108.773855	-17.8972376	L02		
5	M17_I	M17_I	1	1	107.563874	-15.9130075	L02		
6	M9_I	M9_I	1	1	93.553265	18.5316037	L02		
7									

Then we calculate the average of the phage values for the group chosen as the best phased.

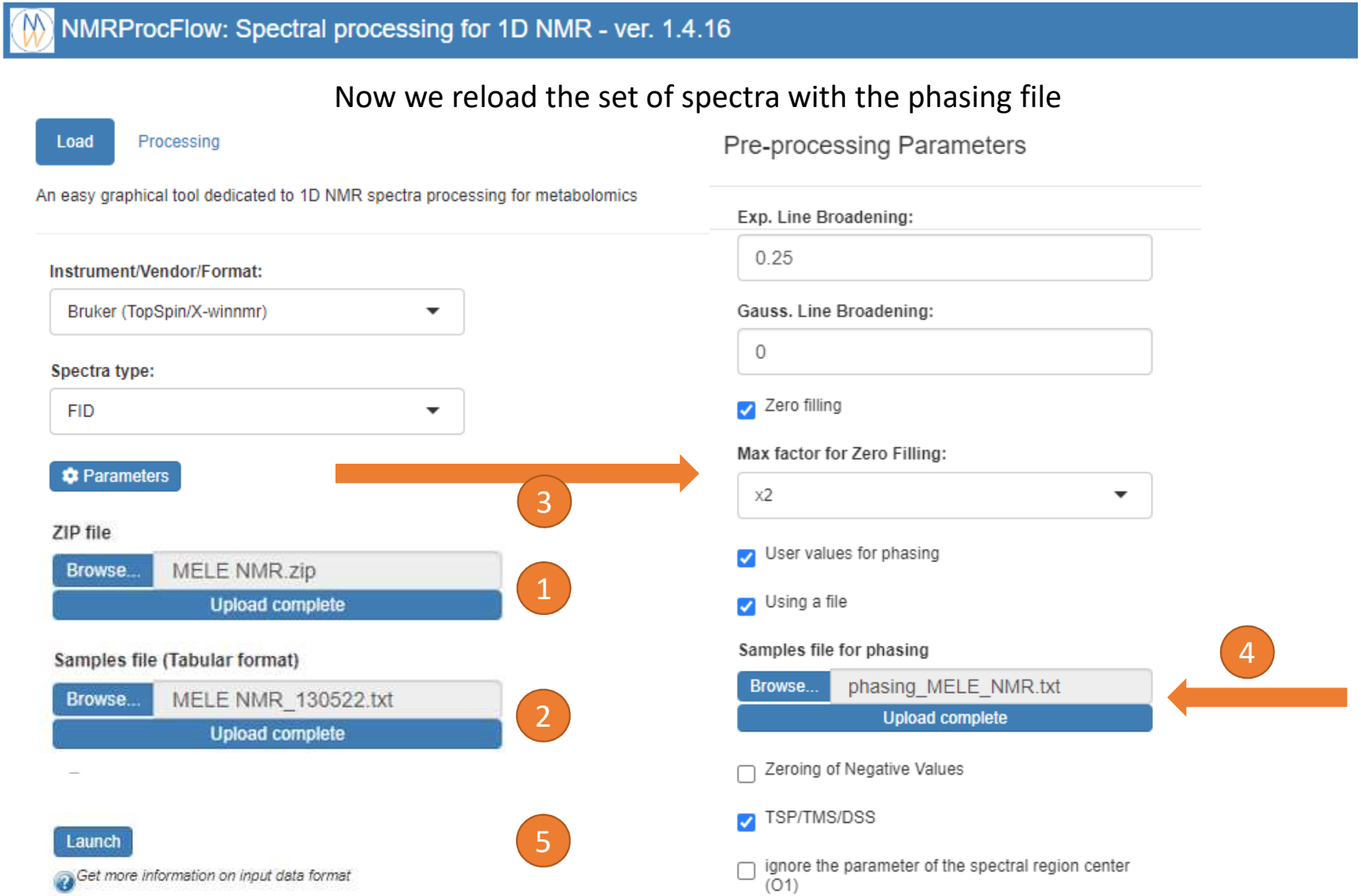
	A	B	C
1	Samplecode	phc0	phc1
2	M1_I	280.94	-20.23
3	M1_II	280.94	-20.23
4	M2_I	280.94	-20.23
5	M2_II	280.94	-20.23
6	M2_III	280.94	-20.23
7	M3_I	280.94	-20.23
8	M3_II	280.94	-20.23
9	M3_III	280.94	-20.23
10	M4_I	280.94	-20.23
11	M4_II	280.94	-20.23
12	M4_III	280.94	-20.23
13	M5_I	280.94	-20.23
14	M5_II	280.94	-20.23
15	M5_III	280.94	-20.23
16	M6_I	280.94	-20.23
17	M6_II	280.94	-20.23
18	M6_III	280.94	-20.23
19	M7_I	280.94	-20.23
20	M7_II	280.94	-20.23
21	M7_III	280.94	-20.23
22	M8_I	280.94	-20.23
23	M8_II	280.94	-20.23
24	M8_III	280.94	-20.23
25	M9_I	109.11	-17.45
26	M9_II	280.94	-20.23
27	M9_III	280.94	-20.23
28	M10_I	109.11	-17.45
29	M10_II	280.94	-20.23
30	M10_III	280.94	-20.23
31	M11_I	109.11	-17.45
32	M11_II	280.94	-20.23
33	M11_III	280.94	-20.23
34	M12_I	280.94	-20.23

...

Thus we can now create the phasing file for samples with 3 columns:

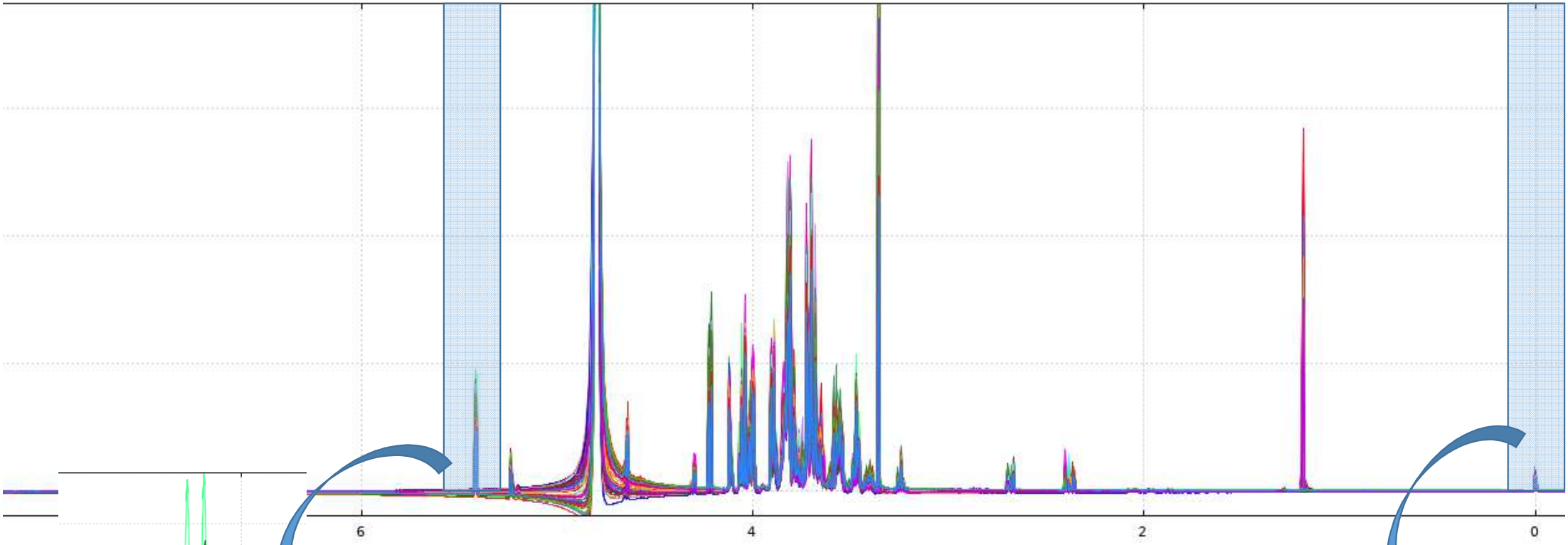
- first column must be the samplecode
- second column must be the zero order phasing value
- third column must be the first order phasing value

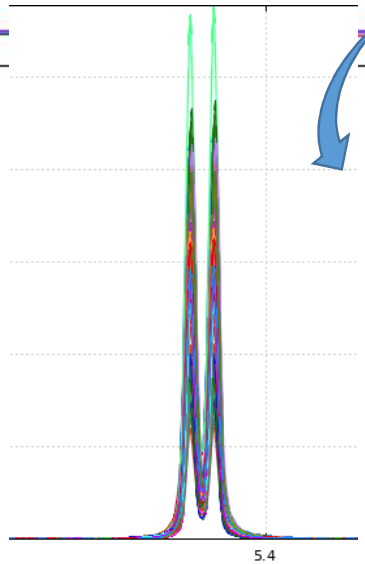
→ phasing_MELE_NMR.txt

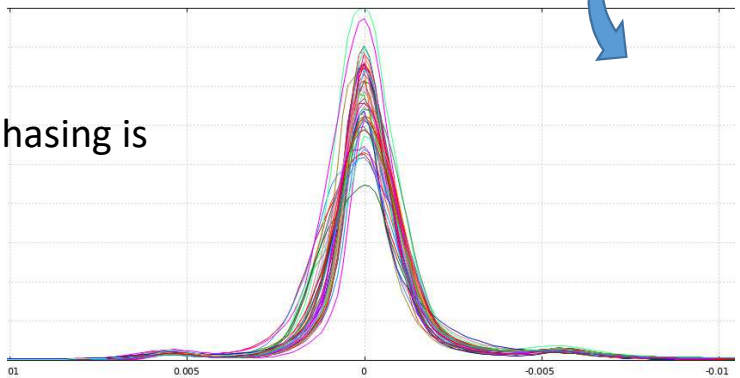


Now we reload the set of spectra with the phasing file

	A	B	C
1	Samplecode	phc0	phc1
2	M1_I	280.94	-20.23
3	M1_II	280.94	-20.23
4	M2_I	280.94	-20.23
5	M2_II	280.94	-20.23
6	M2_III	280.94	-20.23
7	M3_I	280.94	-20.23
8	M3_II	280.94	-20.23
9	M3_III	280.94	-20.23
10	M4_I	280.94	-20.23
11	M4_II	280.94	-20.23
12	M4_III	280.94	-20.23
13	M5_I	280.94	-20.23
14	M5_II	280.94	-20.23
15	M5_III	280.94	-20.23
16	M6_I	280.94	-20.23
17	M6_II	280.94	-20.23
18	M6_III	280.94	-20.23
19	M7_I	280.94	-20.23
20	M7_II	280.94	-20.23
21	M7_III	280.94	-20.23
22	M8_I	280.94	-20.23
23	M8_II	280.94	-20.23
24	M8_III	280.94	-20.23
25	M9_I	109.11	-17.45
26	M9_II	280.94	-20.23
27	M9_III	280.94	-20.23
28	M10_I	109.11	-17.45
29	M10_II	280.94	-20.23
30	M10_III	280.94	-20.23
31	M11_I	109.11	-17.45
32	M11_II	280.94	-20.23
33	M11_III	280.94	-20.23
34	M12_I	280.94	-20.23

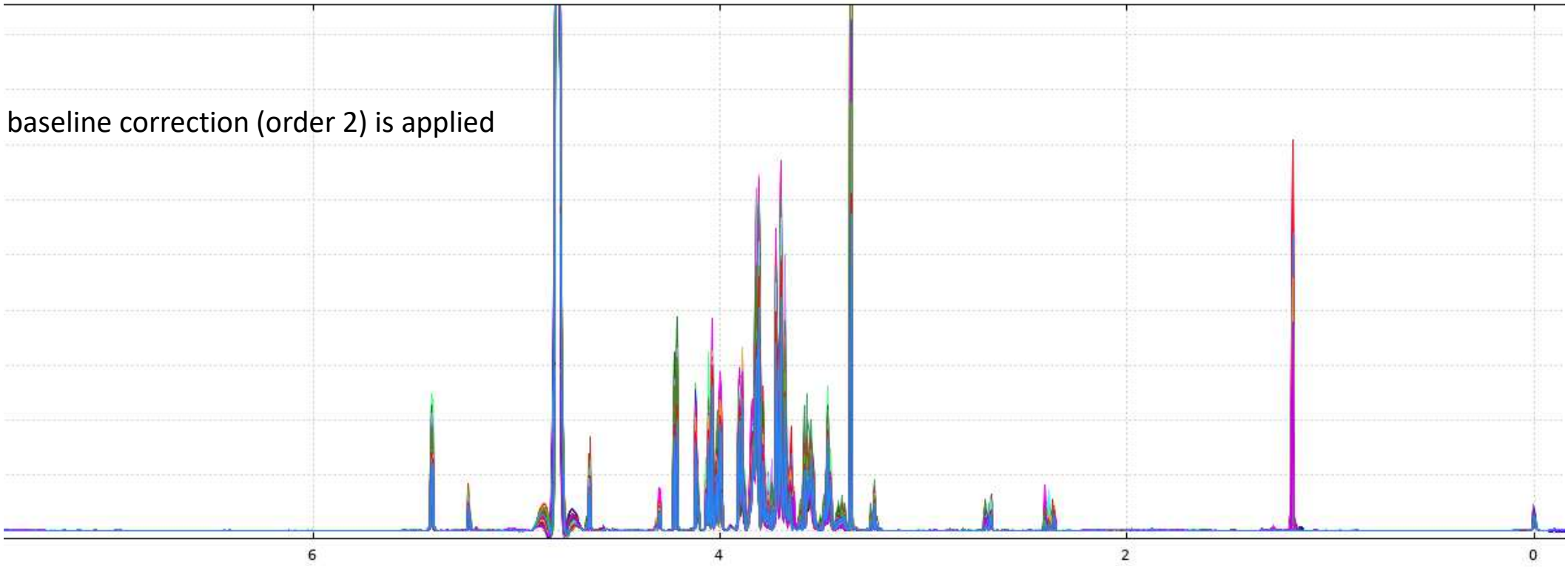






At both ends of the spectra it can be seen that the phasing is correct.

A baseline correction (order 2) is applied



Samples | Processing | Bucketing | Data Export

Processing Type:

- ○ PPM calibration
- ○ Normalisation
- ◉ Baseline correction
- ○ Alignment
- ○ PPM shift
- ○ Zeroing

Type of Correction

Local Correction

noisy PPM range:

8.765 8.415

Restricted PPM range:

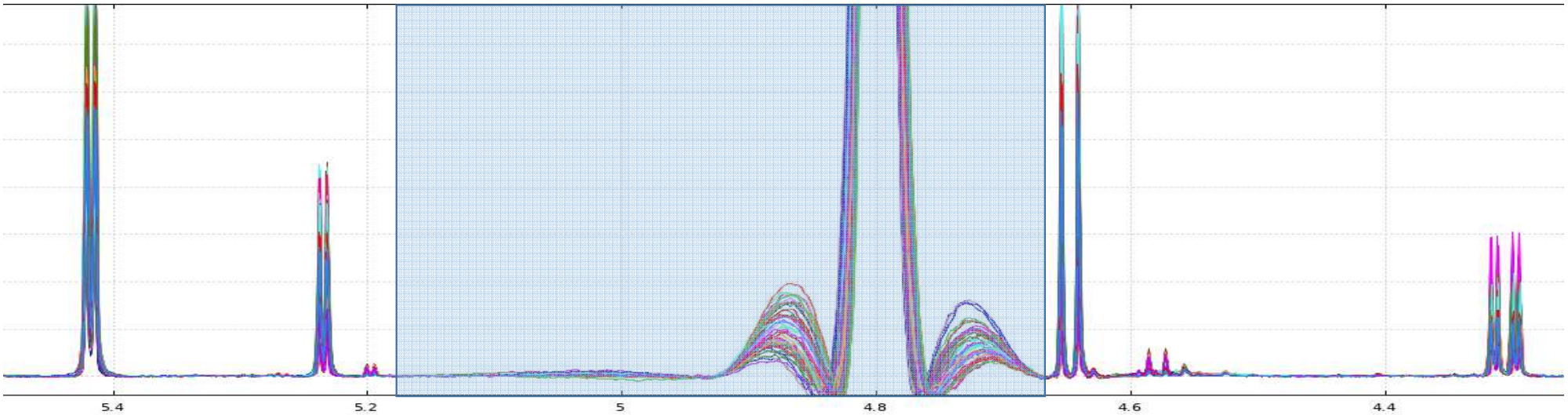
7.968 -0.073

Level of Correction:

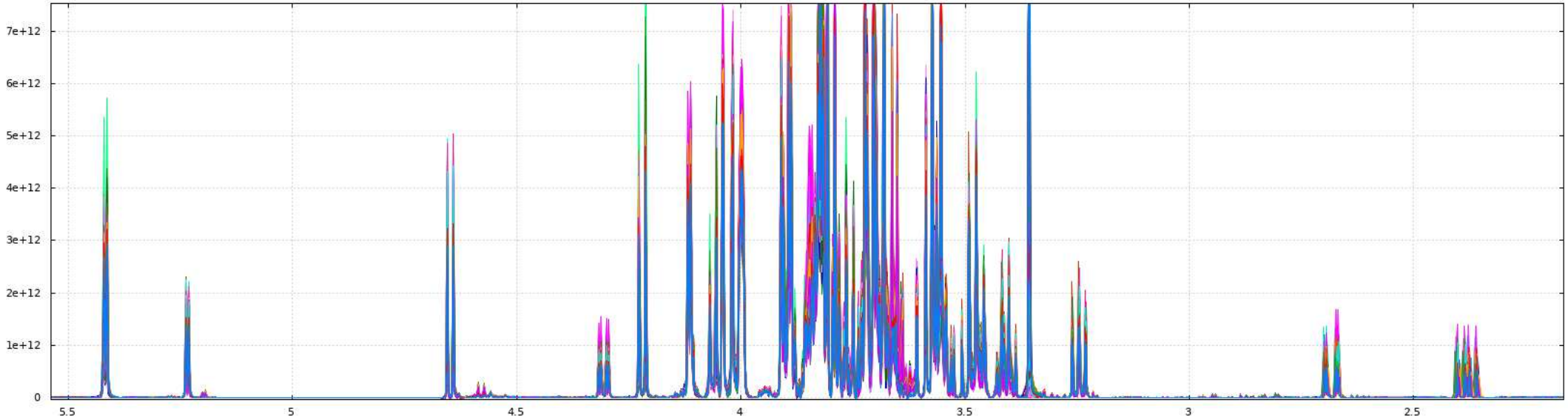
0.3

Order:

2



After zeroing the solvent zone ...



However, if some spectra do not phase correctly using this approach, it is possible to use an online tool to phase manually, and thus retrieve the correct phasing values.

https://pmb-bordeaux.fr/nmrspec/

Of course, this latter approach has to be done for each spectrum individually. Once all the phasing values have been obtained by one or other of the methods, it remains to create the sample phasing file as described above.

Thank you for reading it.

Daniel Jacob – INRAE – UMR1332 – Bordeaux Metabolome – July 2022