

User Guide for the Open Access SYNAPT G2 LC-MS

A. Sample Preparation

1. Weigh **50 – 100 µg** of your compound with a microbalance (e.g. the one in front of the Synapt G2 instrument, Fig. 1) directly into the vial and write down the mass in your laboratory journal.
2. Dissolve your compound in 1 mL of one of the following solvents and seal your vial with a crimper.
 - CH₃OH
 - ACN (acetonitrile)
 - H₂O
 - CH₃OH/CH₂Cl₂ 3:1
 - CH₃OH/H₂O 1:1
3. The sample must be completely dissolved. If the sample does not dissolve completely, try another solvent.
4. The resulting concentration must be **less than 100 µg/mL!** The solution must be diluted if the resulting concentration is higher than 100 µg/mL.
5. Label your vial properly (see Chap. B).



Fig. 1. Mettler AT20 microbalance

B. Rule for Sample Labels

Your samples must be labeled systematically. This is a prerequisite for the automatic transfer of the LC-MS raw data on the processing server. The sample ID consists of three different parts.

1. Two-letter abbreviation for the group name (see table below)
2. "SYN_", which is an abbreviation for the name of the instrument (SYNAPT G2)
3. Individual abbreviation (according to your lab journal entry)

Example: sample 'test01' submitted by Laurent Bigler will be labeled as **IbSYN_test01**

Group Name	Abbreviation
Alberto	al
Bienz	bi
Bigler	lb
Braband	br
Coric	co
Fenner	fe
Freisinger	fr
Gademann	gm
Hamm	ha
Hartrampf	hr
Helbling	hl
Holland	ho
Hutter	hu
Iannuzzi	ia
Juricek	ju
Linden	li
Luber	lb
Nevado	ne
Patzke	pa
Praktikum	a
Rickhaus	ri
Seeger	se
Shoshan	sh
Sigel	sig
Spingler	sp
Tilley	ti
von Rohr	vr
Zelder	zd
Zerbe	ze

Table 1. Group names with corresponding abbreviations.

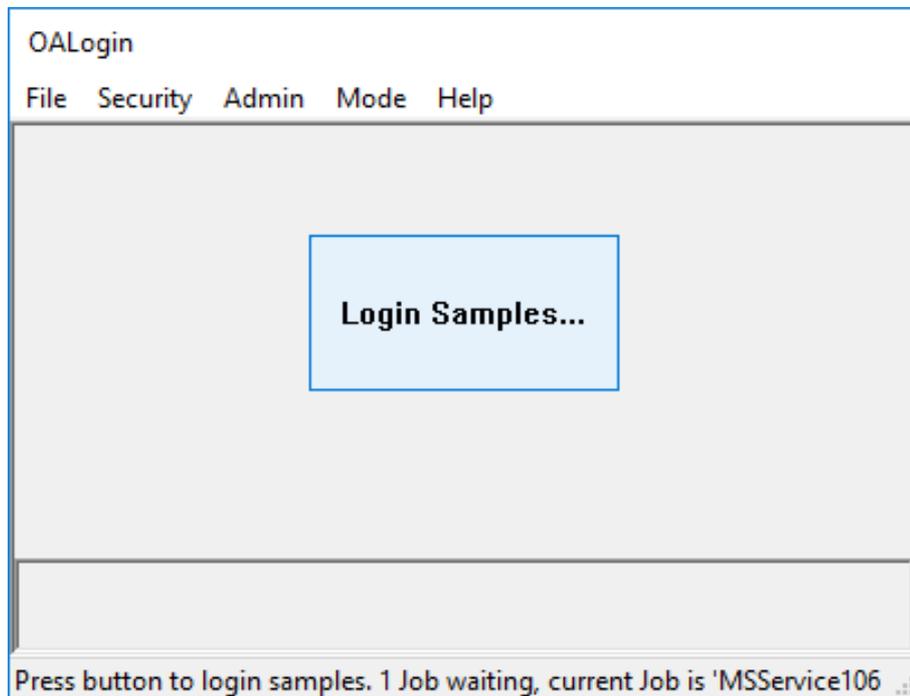
C. Running your sample(s)

The open access methods merge the chromatographic and the MS method. Select one of the available open access methods (OnFlow and LC_isochratic are the fastest ones):

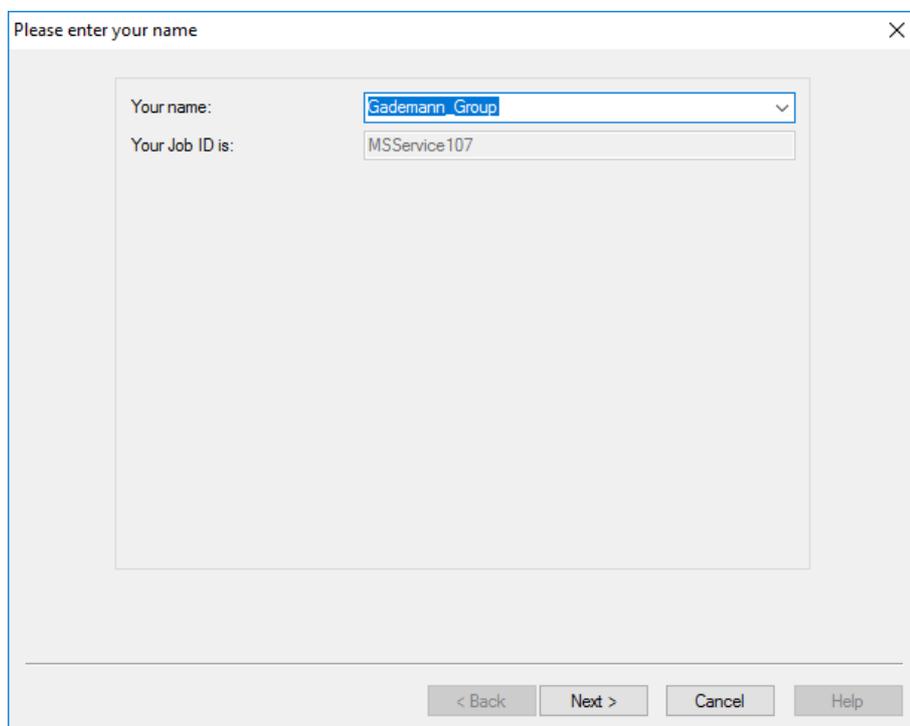
1. OA_OnFlow
2. OA_LC_isocratic_SmallMolecules_C18
3. OA_LC_gradient_SmallMolecules_C18
4. OA_LC_PeptidePolar100_2000Da_C8
5. OA_LC_PeptideApolar100_2000Da_C8
6. OA_LC_Peptide2000_20000Da_C8

- The **OA_OnFlow** method is configured for a 1 μ L on-flow sample injection. There is no HPLC column in use and the mobile phase ($\text{CH}_3\text{OH} + 0.1\% \text{HCOOH}$) is constant over time. The method is configured to measure in the (+)-ESI high-accuracy full scan mode (≤ 3 ppm) and in the mass range m/z 100-2000. This method is dedicated for pure samples or enriched fractions.
- The **OA_LC_isocratic_SmallMolecules_C18** is optimized for a **fast** LC-MS analysis of small molecules (\leq ca. 2000 Da). The HPLC gradient allows a broad separation in less than 5 min with a elution with $\text{H}_2\text{O} / \text{ACN} 20:80 + 0.02\% \text{TFA}$.
- The **OA_LC_gradient_SmallMolecules_C18** is optimized for the LC-MS analysis of small molecules (\leq ca. 2000 Da). The HPLC gradient allows a broad separation in less than 5 min with a gradient elution based on $\text{H}_2\text{O} + 0.02\% \text{TFA}$ (mobile phase A1) and $\text{ACN} + 0.02\% \text{TFA}$ (mobile phase B1).
- The **OA_LC_PeptidePolar100_2000Da_C8** is optimized for the LC-MS analysis of peptides (\leq ca. 2000 Da) with a C8 reversed phase column. The HPLC gradient allows separation in less than 10 min with a gradient elution based on $\text{H}_2\text{O} + 0.02\% \text{TFA}$ (mobile phase A1) and $\text{ACN} + 0.02\% \text{TFA}$ (mobile phase B1).
- The **OA_LC_PeptideApolar100_2000Da_C8** is optimized for the LC-MS analysis of peptides (\leq ca. 2000 Da) with a C8 reversed phase column. The HPLC gradient allows separation in less than 10 min with a gradient elution based on $\text{H}_2\text{O} + 0.02\% \text{TFA}$ (mobile phase A1) and $\text{ACN} + 0.02\% \text{TFA}$ (mobile phase B1).
- The **OA_LC_Peptide2000_20000Da_C8** is optimized for the LC-MS analysis of peptides (between ca. 2000 and 20000 Da) with a C8 reversed phase column. The HPLC gradient allows separation in less than 10 min with a gradient elution based on $\text{H}_2\text{O} + 0.02\% \text{TFA}$ (mobile phase A1) and $\text{ACN} + 0.02\% \text{TFA}$ (mobile phase B1)
- The generated data has to be processed with the MestReNova software (see MestReNova user guide). Make sure that the MS plugins have been activated.

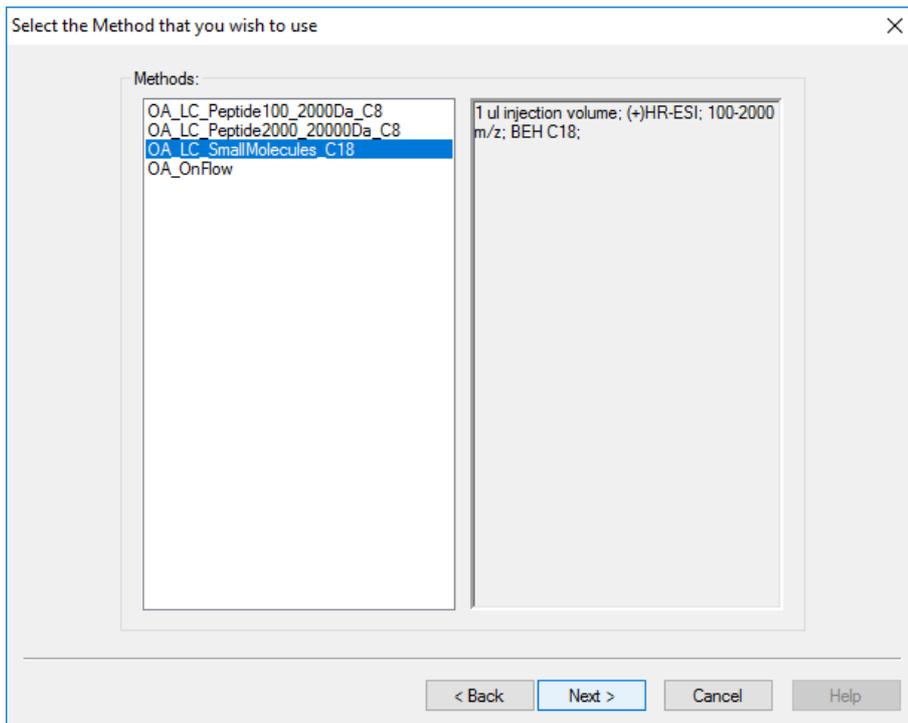
Start the OpenLinks® software.



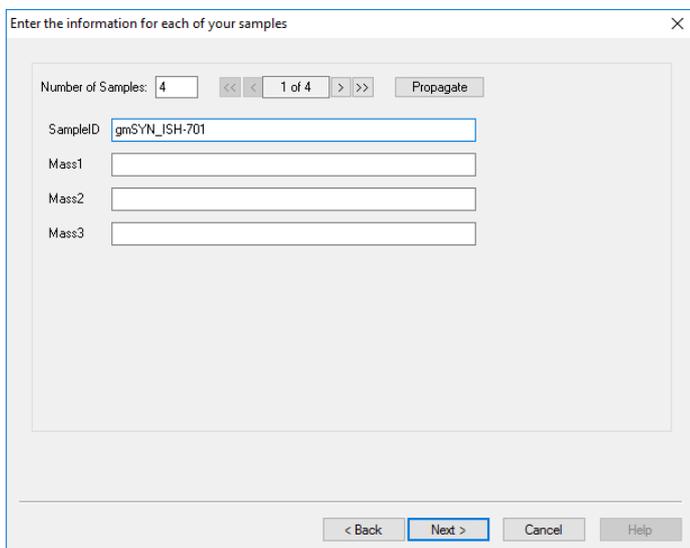
Screenshot 1: Click on “Login Samples...”



Screenshot 2: Select your group name from the dropdown list. Do not change the Job ID

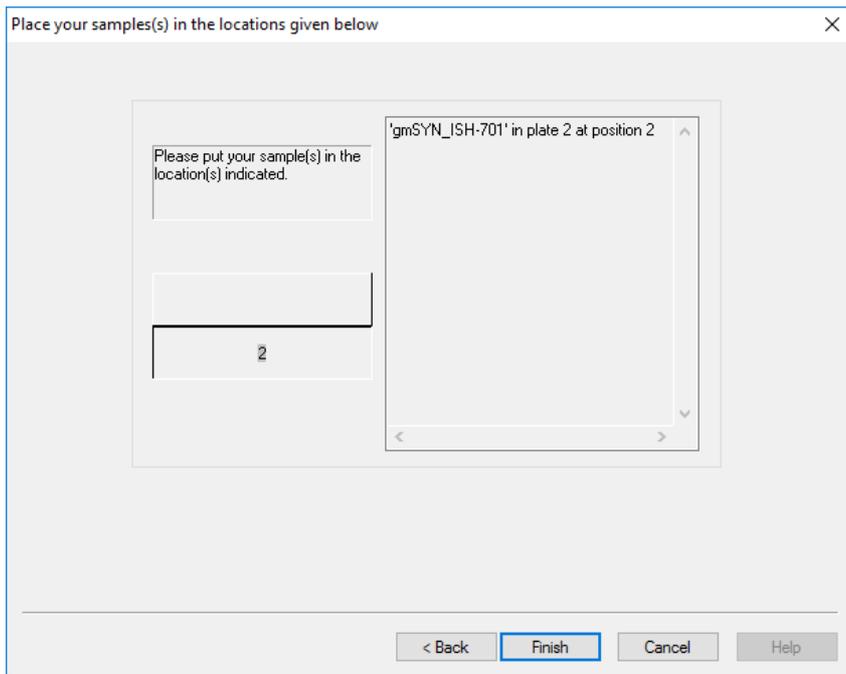


Screenshot 3: Select the appropriate method. In the box on the right side is a short description of the method. You can find a more detailed description of each method on page 2 of this User Guide.



Screenshot 4: Enter the number of samples you want to measure. Give each sample a unique Sample ID according to the nomenclature described under paragraph B. Any existing files with the same Sample ID are overwritten.

Remark: The 'plate 1, position 48' of the QC is fixed. Reinject your sample a second time if this position would have been requested.



Screenshot 5: Place your sample(s) at the indicated location(s) in UPLC sample manager.

Remark 1: Make sure that the sample plate locks in the correct position, otherwise the needle of the UPLC sample manager will be damaged.

Remark 2: Attention, the dialog window closes automatically after a minute or so.

Remark 3: Contact support (Tel 54285 or 54293) if an error message appears (e.g. wrong vial position!).



Click "Finish" to add your job to the queue.

Once the sample is measured, the raw data file is automatically transferred to the corresponding group folder on the server. How you can access your group folder is described in the MestreNova user guide.