

Training on the ZQ Open access MS

Questions?

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STANFORD UNIVERSITY MASS SPECTROMETRY
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Sample Preparation

Sample Preparation: Flow Injection

- ✓ In general, **volatile, low MW protic solvents** are preferred.
 - ✓ **Methanol** is the default solvent. **Acetonitrile** is a good alternative.
 - ✓ If water is required for solubility, **up to 50% water** may be added.

 - ✗ High MW or viscous solvents including DMSO, DMF, and THF should be avoided.
 - ✗ Hydrocarbon solvents, such as hexane and benzene, are not amenable to ESI.

 - Acetone may be used but is not preferred. Even high-grade acetone typically contains contaminants which show up as strong peaks in the MS, and may overwhelm the analyte signal.
 - If the sample contains TFA (e.g. fraction collected from a reverse-phase HPLC run), run by LC-MS. If you wish to run the sample by Flow Injection, remove the TFA by lyophilization or by drying down the sample overnight under high vacuum.
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Sample Preparation: HPLC

- ✓ In general, aqueous solutions are preferred for HPLC analyses. Small amounts of methanol or acetonitrile are acceptable.
 - ✓ All gradients start at 98% Solvent A (0.1% formic acid in water). With these initial conditions, samples with a high concentration of organic solvent can experience breakthrough, where the compound of interest fails to interact with the stationary phase and elutes in the void volume. An aqueous concentration close to the initial gradient conditions is recommended. A smaller injection volume (5 uL) can sometimes compensate for samples with a higher organic solvent content.
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Sample Preparation: Amount

Concentration

- ✓ High quality spectra can typically be obtained from 20-50 μM concentrations (0.05 mg/mL for a compound of MW 1000).
- ✗ Avoid running sample concentrations greater than 0.5 mg/mL, which can lead to the formation of dimers in your spectra, and/or carryover in subsequent runs.

Volume

- ✓ At least 20 μL of sample is needed for a reliable injection by the autosampler. Given a 20 μL sample, the autosampler can aspirate up to 15 μL from the vial. The autosampler uses a 50 μL injection loop, making injections up to 50 μL possible; recommended injection volumes are 25 μL for loop injection, and 5 μL for LC-MS.
- ✓ The required vial can hold as much as 200 μL of sample.

Description	Unit	National Scientific P/N	VWR P/N	Stanford Pricing
Polypropylene limited volume vials, narrow opening	Pk 100	C4013-11	66030-982	\$15.28
Caps for narrow opening vials (8-425)	Pk 100	C4013-60A	66030-420	\$20.52

Sample Preparation

Additional Considerations for Proteins and Peptides

Concentration & Volume

For routine intact mass analysis, the *minimum* amount of protein required depends on the MW of the peptide or protein.

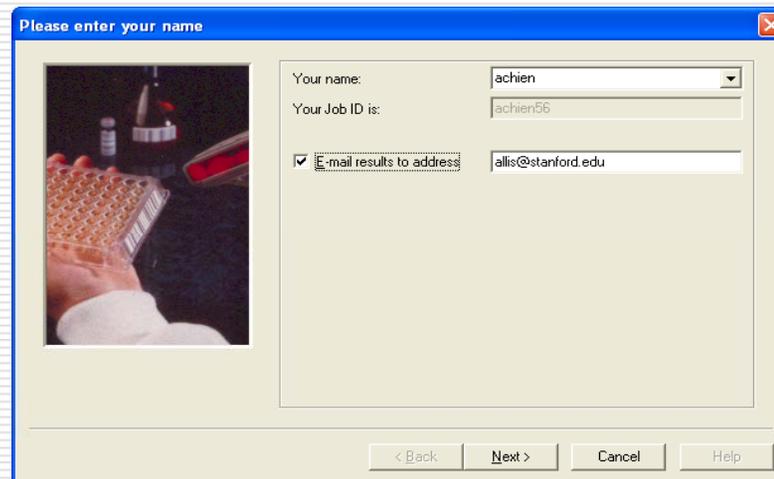
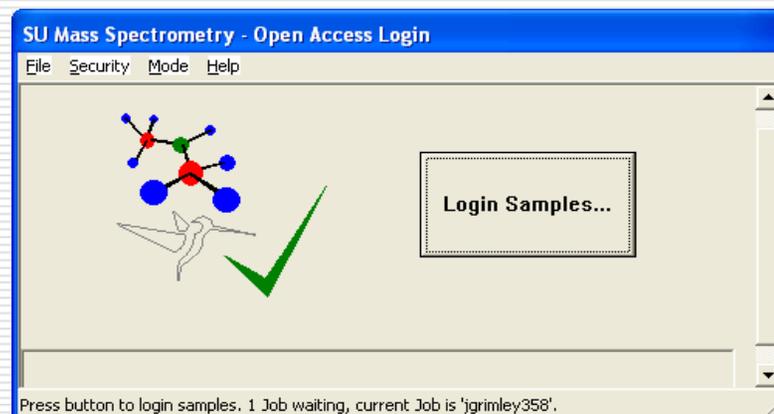
Protein MW	Concentration	Amount
5kD	1uM	25pmol/25uL
20kD	4uM	100pmol/25uL
40kD	8uM	200pmol/25uL
60kD	20uM	500pmol/25uL

Data Acquisition

Data Acquisition

Start with “SU Mass Spectrometry – Open Access Login” window

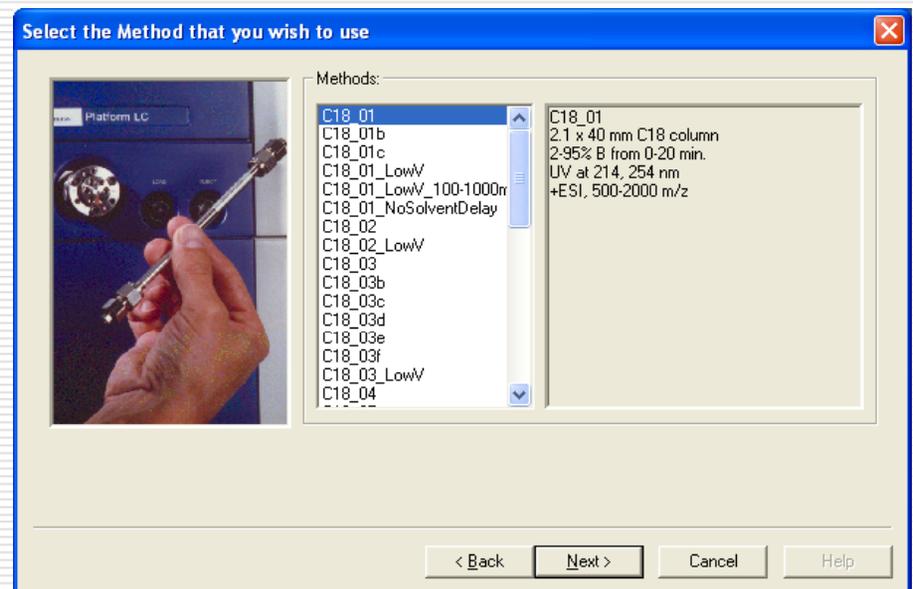
- ❑ Click on the “Login Samples” tab.
- ❑ User name: click the down arrow on the “Your name” box and select your username from the list
- ❑ Emailing the report file to your Stanford email address should be automatically enabled. If not:
 - Click on the “Email results to address” box.
 - Enter your email address (if it is not automatically recalled).
- ❑ *Note: if you receive a report email for a job that you did not submit, forward the report to spectrometry@stanford.edu. Most common cause is a user selecting the wrong username.*



Data Acquisition

Choose a method

- ❑ LC-MS methods are at the top of the menu and loop injection methods are at the bottom.
- ❑ Click on the name of a method to view the parameters in the window on the right.
- ❑ "LowV" refers to methods with a low voltage setting and should be used for samples that fragment at standard ESI settings.
- ❑ The system will default to your last-used method



Data Acquisition

Enter the information for your sample(s)

- ❑ Enter the sample ID (required)
- ❑ The other information is optional:
 - Description
 - injection volume between 5-50 uL (the units are understood)
 - Mass (If you know the monoisotopic masses of your compound of interest, you may enter up to three values for each sample. Extracted ion chromatogram data for these masses will be highlighted in the report.)
- ❑ If there are two or more samples, enter the Number of Samples.
- ❑ Use the arrow key (>) to move from one page to another for each sample.
- ❑ The "Propogate" button copies (and increments, if applicable) info to subsequent pages

Enter the information for each of your samples

Number of Samples: 2 < 1 of 2 > Propagate

Sample ID: test1

Sample Description:

Injection Volume: 25

Mass1:

Mass2:

Mass3:

< Back Next > Cancel Help

Use arrow tabs (>, <) to move from one page to next for multiple sample entries.

Enter the information for each of your samples

Number of Samples: 2 < 2 of 2 > Propagate

Sample ID: test2

Sample Description:

Injection Volume: 25

Mass1:

Mass2:

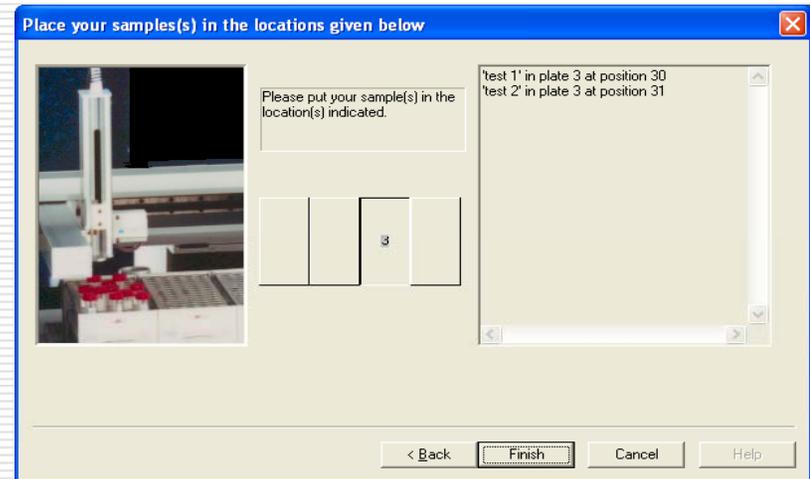
Mass3:

< Back Next > Cancel Help

Data Acquisition

Place sample(s) in autosampler

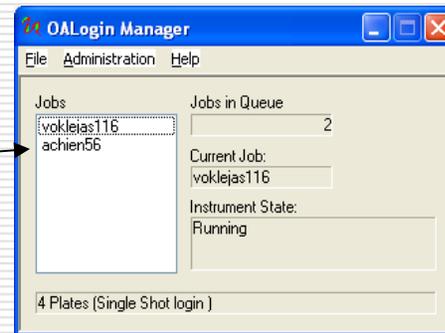
- ❑ First, look at the screen on the HPLC face. If it is not injecting a sample, open the door below the screen to access the autosampler bed.
- ❑ Select the plate number that is assigned to you on the keypad located along the bottom of the screen on the face of the HPLC.
- ❑ The carousel will rotate to the selected plate.
- ❑ Place your samples in the well positions assigned to you.
- ❑ If there is a sample in your assigned position, remove the vial and place it in one of the holding racks. The software will not assign a well position for a sample that has not run yet.
- ❑ Once your vials are in the assigned positions, click on "Finish".



Data Acquisition

Your job will now be listed in the queue window, "OALogin Manager"

- ❑ The analysis will be given a name called a "job" and will be added to the queue window, or "OALogin Manager" window.
- ❑ The job name will be your user name followed by a number (the number corresponds to the number of times you've logged in samples).
- ❑ The job name is the file name for this analysis.
- ❑ Note: The computer is secured such that interaction is possible only with the sample login module.

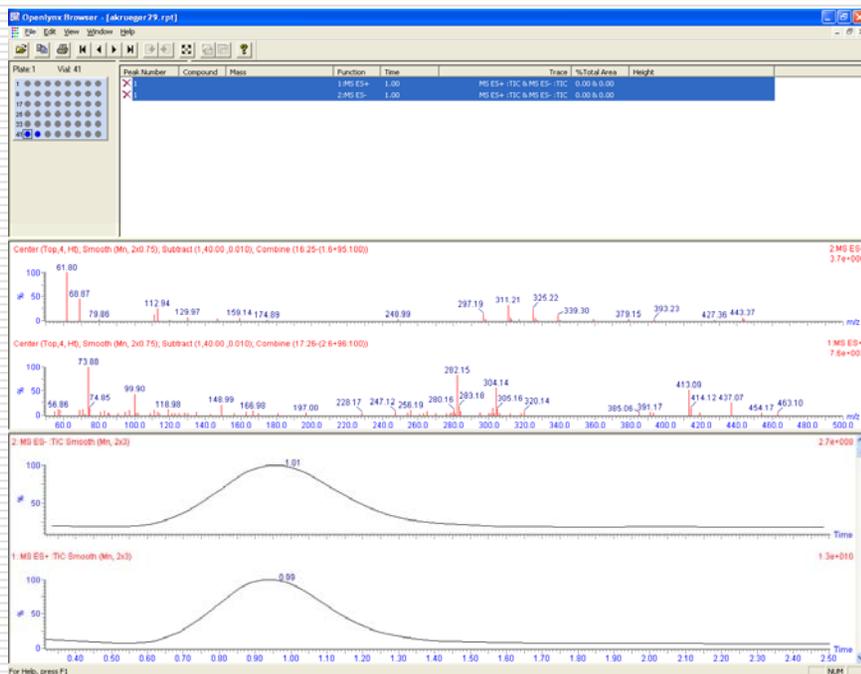


Viewing the data

- OpenLynx Report:
 - A report will be sent to your email when the acquisition is completed.
 - OpenLynx software must be installed on your PC computer in order to view the report (obtain this from SUMS).
 - The report name will be the same as the job name for this analysis.

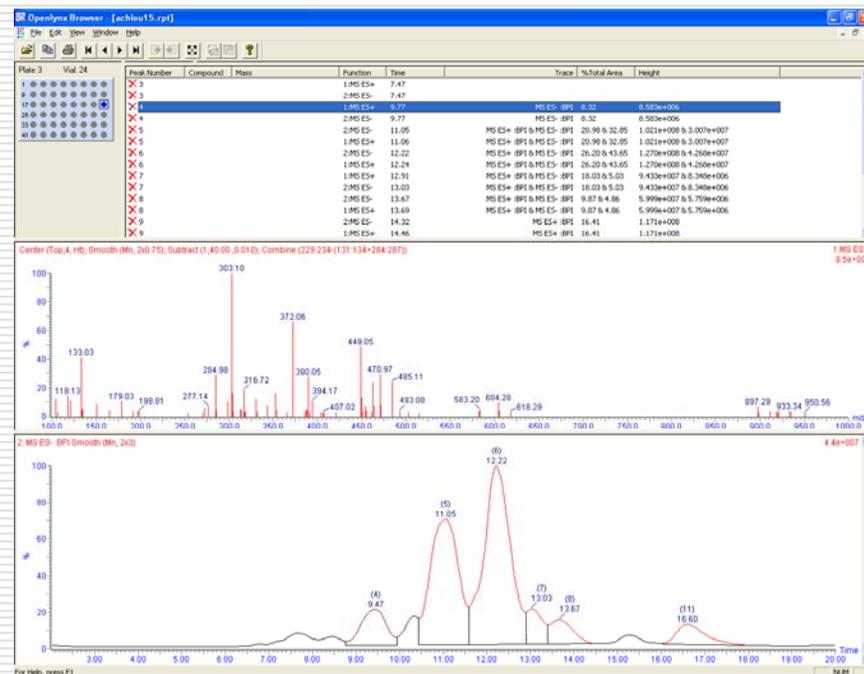
 - Raw (full) data file:
 - Data files are transferred to the data analysis computer once per hour, 24 hours a day, at approx. xx:30.
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The Open Lynx Report format



□ Loop injection report:

MS data is recorded from only one time point in TIC, 1.00 minute, the amount of time it takes for the sample to go from the injector to the detector.



□ LC-MS report:

MS data is recorded from multiple time point in TIC, where signal in the chromatogram is above the baseline threshold. Clicking on each retention time listed will update the mass spectrum for the corresponding RT.

FAQs on Data Interpretation

- Expected mass accuracy for a single quadrupole mass spectrometer:
 - $MW < 2000 \pm 0.2 \text{ Da}$
 - $MW > 2000 \pm 0.01\% * MW$
- Common electrospray-MS adducts, where M = the monoisotopic molecular weight of the neutral species.

Positive electrospray (ES+)	Negative electrospray (ES-)
$M+H^+ = M+1$	$M-H = M-1$
$M+Na^+ = M+23$	$M-H+\text{formic acid} = M+45$

- Samples resulting in mass spectra with an intensity $> 5e7$ are too concentrated, and more dilute solutions should be run in the future. The detector saturates at $1.3e8$.
 - To learn more about mass spectrometry check out <http://mass-spec.stanford.edu/Tools&Links.html#Tutorials>.
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Data analysis workstations

- ❑ Data files are transferred to the data analysis computer once per hour, 24 hours a day, at approximately xx:30
- ❑ Both data analysis computers are running MassLynx & have desktop shortcuts to the data folder
- ❑ Your data belongs to you
 - You are responsible for backing up your own data
 - Data are stored here for only a few months

If you need to deconvolute protein data:

- use the “deconvolution” Windows login in order for MaxEnt1 to work properly – contact Pavel for details
- Please **exit** MassLynx and log out of the Windows account when you are done
- Doing so will allow others to use the MassLynx program, since the computer can only run on instance of MassLynx at a time

If that doesn't work...

□ Go ask Theresa

- tmcl@stanford.edu
- 650.725.9769



□ Or Allis

- allis@stanford.edu
- 650.723.0710



Copies of this training material may be downloaded from the SUMS website at

<http://mass-spec.stanford.edu/Instruments-OpenAccess.html>

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