ORIGAMI: A Software Suite for Activated Ion Mobility Mass Spectrometry Applied To Multimeric Protein Assemblies

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ORIGAMI is designed to provide intuitive graphical interface for routine analysis of mass spectrometry and ion mobility mass spectrometry datasets. The program was designed with activated IM-MS (or CIU) datasets in mind, in particular to speed up the extraction of data, processing and improve the visualisation methods.

Disclaimer

Citation

If you use either of the components of ORIGAMI, please consider citing it in your work. Here is a link to the paper on the IJMS website.

Link: https://doi.org/10.1016/j.ijms.2017.08.014

Preface

ORIGAMI^{MS} works by interfacing MassLynx and Waters Research Enabled Software (WREnS) to carry out a typically tedious task of increasing the collision or cone voltage prior to ion mobility separation. The program works by executing a pre-compiled C# code alongside normal acquisition, however the C# (WREnS code) modifies some of the DC potentials on the instrument, taking control away from MassLynx. In ORIGAMI^{MS} case, we only control three DC potentials, the DRE lens which controls the attenuation of the ion beam, the sample cone voltage (SAMPLE_CONE_VOLTAGE_SETTING) and trap collision voltage (SOURCE_BIAS_SETTING). These are modified on-the-fly, as the acquisition continues, to reflect the user settings. The latter two can be controlled in voltage range of 0 to 200 V, which provides sufficient activation energy to unfold and fragment most compounds.

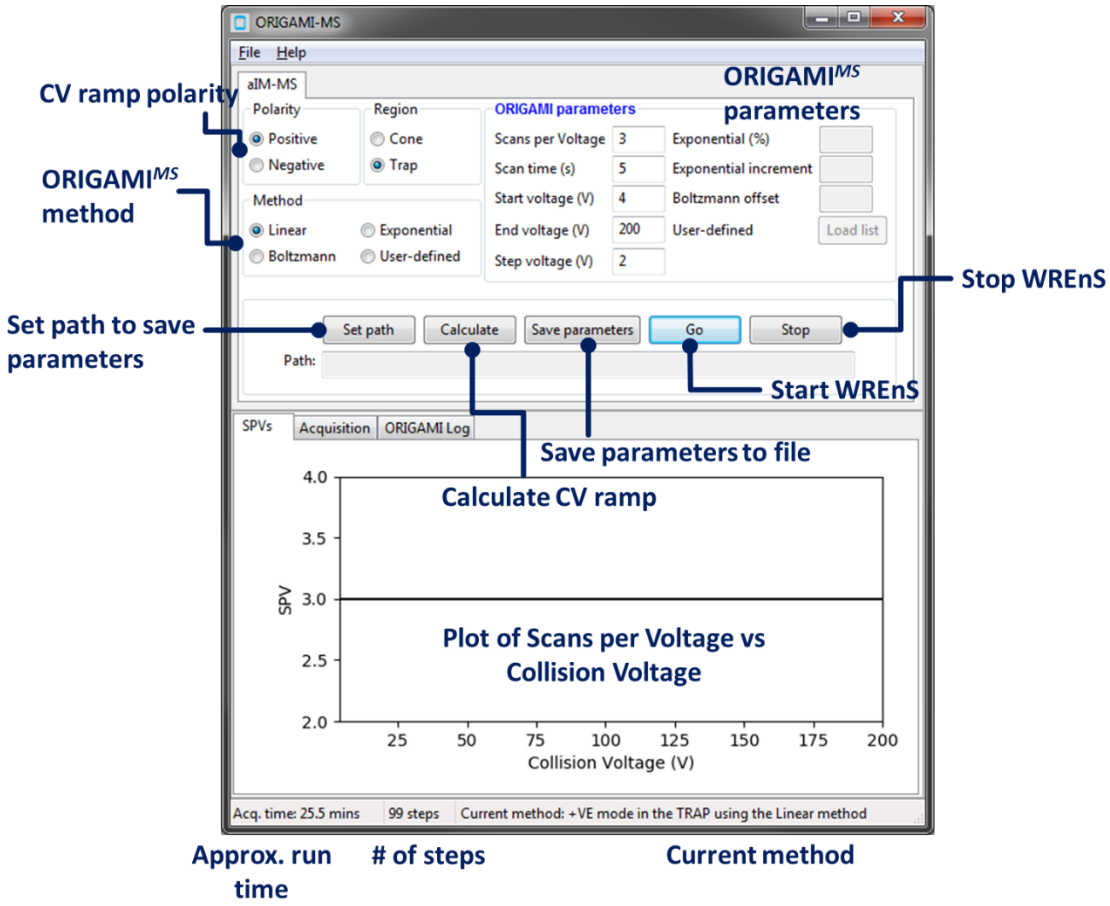
How to install ORIGAMI^{MS}

Actually, there is no actual installation. Simply copy the zipped folder onto your machine, unpack it and that's it. To start ORIGAMI^{MS} you simply double-click on the **ORIGAMIMS.exe** (has logo).

Pre-requisites

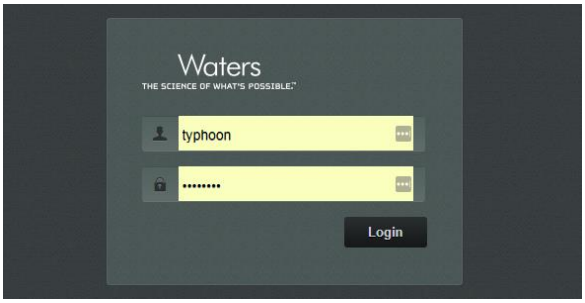
1. You have read this guide.
2. Make sure you install WREnS on your instruments PC. See Waters Corp. instructions.
3. Make sure you compile each script in WREnS GUI before trying to run ORIGAMI^{MS}. If you are not sure how to do this, then have a look at **How to compile ORIGAMI scripts** below.
4. Check that your version of WREnS has the executable "**ScriptRunnerLight.exe**". If it does not, then please contact Waters Corp. for the newest version of WREnS.
5. Make sure you have ORIGAMI^{MS} on your instrument PC.
6. Make sure you have access to PC with Driftscope, otherwise you will not be able to analyse MassLynx files. You will still be able to process and visualise text files.

User Interface

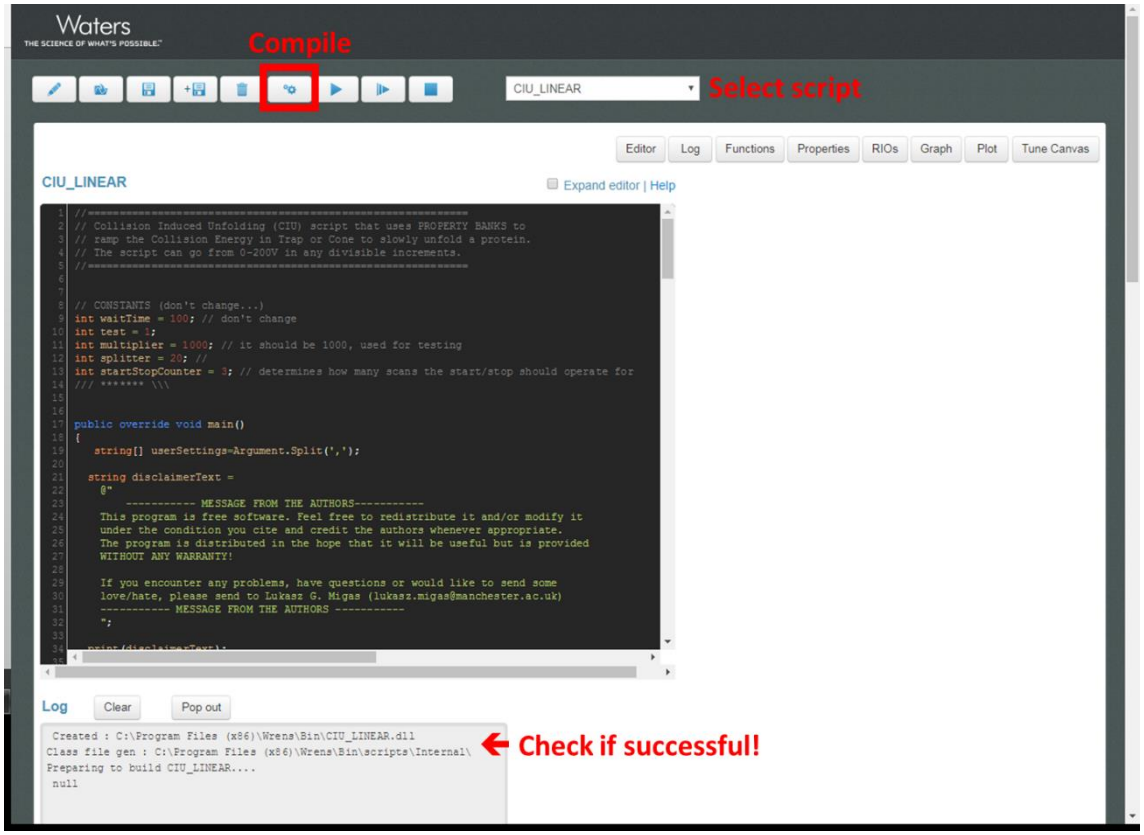


How to compile ORIGAMI scripts

1) Copy C# scripts onto your instrument PC, typically:
   C:\Users\Administrator\Documents\Wrens\Scripts
2) Start WREnS from Start → Wrens
3) A browser window/tab should open with this looks:



4) Login and select the script to compile.



5) Compile the script using the  button and repeat the action for each file.
6) If successful, the log at the bottom of the screen should say "Created: C:\...."
7) **If not, please contact Lukasz G. Migas at lukasz.migas@manchester.ac.uk**

Note: If you copied the scripts into the folder after you opened WREnS, you might have to refresh the window or restart WREnS.

Basic usage

ORIGAMI^{MS} can operate in three ways (two are showcased and provided in the initial release, v1.0):

- **Using Graphical User Interface – the easiest method to get started**
  1. Open ORIGAMI^{MS}.
  2. Select polarity, activation region, ORIGAMI^{MS} method and fill-in appropriate parameters (text boxes are activated and deactivated depending on the method you selected).
  3. Calculate your parameters using the  button and check that your code was generated correctly (have a look in the ORIGAMI Log tab in the GUI).
  4. Go to MassLynx tune page and start acquisition.

  Note: You can either set the acquisition time in MassLynx (make sure you add a couple of minutes to the run time) or you can come back to it after the pre-defined amount of time. See **Troubleshooting** for more details.

  Note: Make sure your scan time is the same as that you set in the GUI.

5. Go back to ORIGAMI^{MS} and press  button in the GUI. If your setup was correct, a new window should appear.
6. Let it do its thing and come back when the acquisition is finishing/ed.
7. Analyse your data in ORIGAMI^{ANALYSE}.

Note: If you need to cancel the run, just press on the  button, stop the acquisition and reinitialise MassLynx. See in **Troubleshooting** for more details.

- **Using the command line – still easy but also easier to make a mistake**
  1. You can either run Command Prompt from within the **C:\Program Files (x86)\Wrens\Bin** folder or from anywhere on your instrument PC.
  2. Open Command Prompt by typing 'cmd' in the title bar of the folder.
  3. If you are running from within **C:\Program Files (x86)\Wrens\Bin** then the general rules are:

     ScriptRunnerLight.exe SCRIPT_NAME.dll PARAMETERS

  4. If you ae running from somewhere else on your system, then you have to append the WREnS path to the name:

     "C:/Program Files (x86)/Wrens/Bin/ScriptRunnerLight.exe" SCRIPT_NAME.dll PARAMETERS

  5. You can stop the script at any time by pressing **CTRL+C** on your keyboard.

  Note: If you exit the program prematurely, make sure you run the CIU_RESET.dll script afterwards, otherwise the script will be running in the background.

  6. The actual commands are (shown without the WREnS path).

Linear method:

ScriptRunnerLight.exe CIU_LINEAR.dll TRAP,POSITIVE,3,5,4.0,200.0,2.0,25.5

Parameters: Region – TRAP; Polarity – POSITIVE; SPV – 3; scan time – 5; start V – 4; end V – 200; step V – 2V; approx. run time – 25.5 (can be any number!)

Exponential method:

ScriptRunnerLight.exe CIU_EXPONENT.dll TRAP,POSITIVE,3,5,4.0,200.0,2.0,20.0,0.03,92.75

Parameters: Region – TRAP; Polarity – POSITIVE; SPV – 3; scan time – 5; start V – 4; end V – 200; step V – 2V; exponential% - 20; exponential Increment – 0.03; approx. run time – 92.75 (can be any number!)

Boltzmann method:

ScriptRunnerLight.exe CIU_FITTED.dll TRAP,POSITIVE,3,5,4.0,200.0,2.0,50.0,61.25

Parameters: Region – TRAP; Polarity – POSITIVE; SPV – 3; scan time – 5; start V – 4; end V – 200; step V – 2V; Boltzmann offset – 50; approx. run time – 61.25 (can be any number!)

User-defined method:

ScriptRunnerLight.exe CIU_LIST.dll "TRAP,POSITIVE,5, [3 3 3 3 3 3],[4 6 8 10 12 14]

Parameters: Region – TRAP; Polarity – POSITIVE; scan time – 5; start V – 4; end V – 200; step V – 2V; list of SPVs – [3 3 3 3 3 3]; list of CVs – [4 6 8 10 12 14]

Note: This is probably the hardest method to get right. The parameters must start with quotation mark (") and values cannot be separated by anything other than comma, apart from the values inside the square brackets ([ ]) which have to be separated by single space. Of course, the lists of SPVs and CVs must of the same length.

Note: In general, the values inside the PARAMETERS section of the code must not be separated by anything other than a comma (,).

Good practice: Make sure you save the ORIGAMI^{MS} parameters in your notebook, in the header of the MassLynx .raw file or as configuration file inside the MassLynx .raw file. This way, you will make your life a lot easier and your analysis will be swift and pain-free.

Troubleshooting

The calculated acquisition time does not match the run time. Why is that?

By default, each of the ORIGAMI^{MS} methods acquires a couple of extra scans at the start and end of the activation period. The 'reporter' regions are approx. 3 scans each, however at the end of the run, additional couple of scans are added to complete the acquisition loop. Typically, the number of extra scans is equal to the number of scans per voltage for the last activation voltage. In some cases, this can be quite large (*i.e* if you are using any of the non-linear methods and your gradient is steep).

This behaviour might change in future as it is not strictly necessary.

Why does my scan time have to match in MassLynx and ORIGAMI^{MS}?

The reason for this is quite simple. Each of the scripts uses something called *Property Banks* which are available in WREnS. These operate by pinging and setting parameters based on scan numbers. If the scan time is 5 s in MassLynx but only 1 s in ORIGAMI^{MS} then by the time single scan has finished in MassLynx, ORIGAMI would have thought that 5 have passed and therefore, the activation ramp would be completely ignored as the applied voltages would be in a mess. Alternatively, if the scan time in ORIGAMI is 5 s but only 1 s in MassLynx, then 5 scans would have passed in MassLynx but the activation ramp would have only progressed by one scan. The second scenario is less 'bad' since for each one scan in ORIGAMI we would have 5 scans in the MassLynx .raw file so in during analysis, we would just have to multiple the number of scans per voltage by the division of (set scan time/real scan time) – bear in mind, that this would only work if the values were divisible! In the first scenario, there would be no possible recovery, as there would not be sufficient number of scans to perform any analysis on, since for each one MassLynx scan, there could have been multiple activation steps taken. **Ideally, both cases should be avoided!**

Why should I save my parameters?

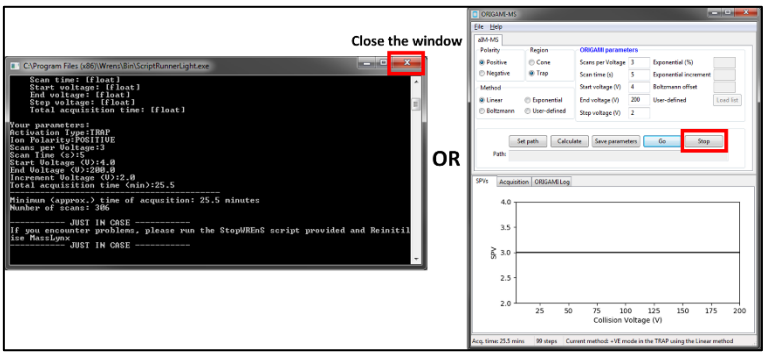
At the moment, ORIGAMI^{MS} nor ORIGAMI^{ANALYSE} are clever enough to figure out the parameters from the MassLynx .raw file. If you save your parameters, your analysis will be very straightforward. We recommend for you to use the 

button and save the 'origami.conf' file directly into the MassLynx .raw folder as when you open it in the analysis software, those parameters will be automatically loaded. Otherwise, just write them down in your notebook.

I have started an acquisition but would like to cancel it. What should I do?

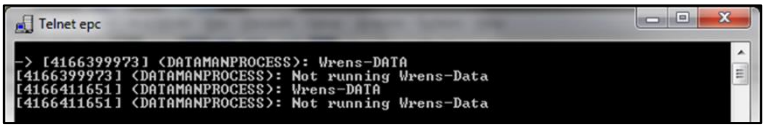
It is quite simple.

1) Press the Stop button in the GUI or close the 'Command Prompt' window that just opened.

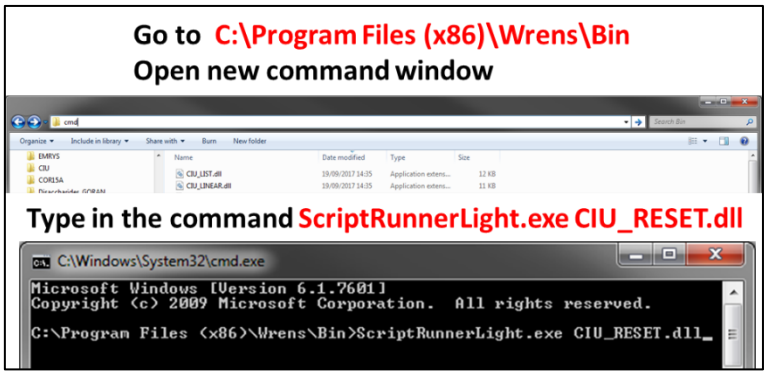


2) Check that WREnS code is not running in the background. Open new Command Prompt window (Start → type in **telnet epc**). Wait for the ticker to change. It should say:

"Wrens-DATA
Not running Wrens-Data"



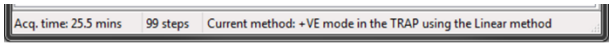
3) If it does not say that, you should open WREnS directory (typically: **C:\Program Files (x86)\Wrens\Bin** and open new Command Prompt window from within the address tab and execute the following command.
ScriptRunnerLight.exe CIU_RESET.dll



4) Stop acquisition in MassLynx GUI.
5) In the same window, click on Acquire → Reinitialise…

I typed in all parameters but I get the message "Are you sure you filled in correct details or pressed calculate". Why?

1) Check what the 'ORIGAMI Log' tab tells you.
2) Make sure you typed in correct values.
3) Make sure you pressed on Calculate button. Check the status bar to see whether your 'code' was computed.



Usually a good indication if parameters were computed correctly

I have definitely filled in all parameters and no message appear, just a quick flash of something (perhaps command window?). Why is that?

There couple be a couple of reasons.

1) WREnS cannot find your script. Have you put the .dll scripts in 'C:\Program Files (x86)\Wrens\Bin'?

   Try putting script dlls in that folder. You will need administrator privileges.

2) WREnS cannot read your script. Have you tried compiling each .dll script yourself?

   Try to compile each script before proceeding.