

# Crommy User Manual version 1.0.0

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Release history: March 2024 Software version: Crommy 1.x For Research Use Only. Not for use in diagnostic procedures.

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# System Requirements

System	Minimum requirements
Hardware	3.4 GHz dual-core processor
	1 6 GB RAM
	1TB hard drive
Software	Windows 10 64-bit operating system or Windows 11 64-bit operating
	system
System settings	To run processing workflows with online library database, the computer
	must have unblocked access to the library databases on the Internet.

### Installation

#### Download the latest Crommy version from <u>www.msforge.it/software</u> and execute for installation.

cense Agreement		$\rightarrow$
Please read the following important information before continuing.		(10
Please read the following License Agreement. You must accept the term continuing with the installation.	ns of this agreement b	efore
SOFTWARE LICENSE AGREEMENT		
BY CLICKING THE "ACCEPT" BUTTON OR OPENING THE PACKAGE, YOU BOUND BY AND ARE BECOMING A PARTY TO THIS SOFTWARE LICENSE €œAGREEMENTâ€). IF YOU DO NOT AGREE TO ALL OF THE TERMS THE "DO NOT ACCEPT" BUTTON. IN SUCH CIRCUMSTANCES YOU ARE THE SOFTWARE AND YOU SHOULD CONTACT US IMMEDIATELY TO DE	ARE CONSENTING TO AGREEMENT (â OF THIS AGREEMENT, NOT PERMITTED TO U TERMINE YOUR OPTIO	BE CLICK JSE NS.
THE PARTIES		
<ul> <li>a) The parties to this Agreement are MS Forge S.r.l.s. and the Custom- licensor of the MSInside version 1.0 software that is the subject of this</li> <li>€ ). The "Customer" is the person or organization that orders, uses ar fee, if any, for the Software.</li> </ul>	er. MS Forge S.r.l.s., Agreement ("Soft nd pays the applicable	the wareâ license
b) In granting this license to the Customer, MS Forge S.r.l.s. may also Information Partners "Information Partners" are entities that have lice	be acting on behalf of nsed to MS Force S r	ام
◯ I accept the agreement		
O I do not accept the agreement		
	Next	Cance

Read the licence agreement and accept by selecting "I accept the agreement" and then click on the Next button for continuing the installation.

Setup - Crommy version 1.0.0	-	_		×
Select Destination Location				FY
Where should Crommy be installed?				HOM
Setup will install Crommy into the following folder.				
To continue, click Next. If you would like to select a different folder, click Bro	wse.			
C:\Users\user\AppData\Local\Programs\Crommy		E	rowse	
At least 336.0 MB of free disk space is required.				
Back	Nex	t	C	ancel

You can modify the installation folder (optional) and than click on Next to continue the installation.

Setup - Crommy version 1.0.0			_		×
Select Additional Tasks					FY
Which additional tasks should be p	performed?			(	HOW
Select the additional tasks you wo	ould like Setup to perform	while installing Crommy,	then clic	k Next.	
Additional shortcuts:					
🔽 Create a desktop shortcut					
		Back	Next		ancel

You can now select to create a desktop shortcut. Click Next to move on the summary page.

🙀 Setup - Crommy version 1.0.0 —		×
Ready to Install Setup is now ready to begin installing Crommy on your computer.		
Click Install to continue with the installation, or click Back if you want to review or change a	ny settings	
Destination location: C:\Users\user\AppData\Local\Programs\Crommy Additional tasks: Additional shortcuts: Create a desktop shortcut		
4	Þ	•
Back Install		ancel

Here you can click "Install" to complete the installation.

# **Crommy** activation

### After starting Crommy, the following screen will appear:



Click «Ok».

Enter a valid email and a valid Licence Key.

Press «Verify»

A new popup will appear confirming successful registration.



I you don't own a valid licence, you can send us a licence request on <a href="https://www.msforge.it/contact/">https://www.msforge.it/contact/</a> asking for private, public or demo license.

### Crommy top bar

Four buttons are present on the top-right of the app





Setting icon display the registration space if the software is not registered yet, otherwise by clicking on setting it will appear a page with the

button «User manual». By clicking on the button, this manual will open automatically.



By clicking this button, Crommy will minimize.



By clicking this button, Crommy will maximize in the screen.



By clicking this button, a pop-up will open. By clicking «Yes» the app will close.



# Crommy left bar

Crommy is divided in 5 main pages linked to the relative icon. Moving the mouse of each of the icon will reveal the icon name.

Full icon name can be displayed by clicking on



After clicking the space next to the icons will expand showing the name of each icon.

		1.1
<	Hide Menu	Н
6	Home	С
C		р
<u>iii\</u>	Library	р
		L
पि	Annotation	h
20	late and in a	li
Z	Integration	n
		A
		D
		S
		11
		D
		С

Hide the icon names.

Home

Contain the tools for starting a new project or for loading already analyzed project.

Library

Include all common and user spectral libraries and tools for libraries management.

Annotation

Display compound annotation and relative scores.

Integration

Display the peak area for each detected compound.

### Home

Clicking on Home button will open a new window on the left side.



### Start new project

After clicking «New Project» the central screen will change by visualizing the Workflow manager.

Ξ	Cr	om	my						¢		ı ×	
â					Work	flow	mar	ager				
IIIN				Identification dataset					Quantitation dataset			
				File name	Туре				File name	Туре		
पि		1	Х	C:/Users/Giuseppe/OneDrive - CNR/test/chrom_1.raw	Full scar	•	1	Х	C:/Users/Giuseppe/OneDrive - CNR/test/chrom_1.raw	Blank	-	
2.0	1	2	Х	C:/Users/Giuseppe/OneDrive - CNR/test/chrom_2.raw	DDA	•	2	x	C:/Users/Giuseppe/OneDrive - CNR/test/chrom_2.raw	QC	-	
22		3	Х	C:/Users/Giuseppe/OneDrive - CNR/test/chrom_3.raw	DIA	•	3	Х	C:/Users/Giuseppe/OneDrive - CNR/test/chrom_3.raw	Sample	-	
									Select Raw Files			
				Select Raw Files			Smoothing					
	Target compounds											
				Validate					Exit			
	By: N	/IS F	orge								0.1.0	

On top, two table are available.

Identification dataset table can be populated by selecting chromatographic files (.raw or mzML format) and specify the analysis type (Full scan, DDA or DIA). There are no restrictions to the number of files or the combination of different approaches e.g. DDA and DIA. Based on the uploaded dataset, Crommy will automatically select the workflow optimized for the analysis. Automatic peak detection and annotation will be performed on this selected chromatograms. When running targeted analysis only, this table can be left blank.

Quantitation dataset table can be populated by selecting chromatographic files (.raw or mzML format). This dataset may or may not contain files from identification dataset.

### Target Compound table

				Target compo	unds			
		Compound name	Molecular Formula	Adduct	HMDB	InChlKey	m/z	retention time
1	Х	Glucose	C6H12O6	Н-			179.05611	7
Glucose		С6Н12О6 7	H-	▼ HMDB	InchlKey	Add	Import	Export

If selected, this session will expand giving the possibility to include compounds as conventionally done in targeted analysis.

Compound name and Chemical formula are mandatory fields.

Type of adduct can be selected (default is +H). HMDB ID and InChlKey are not mandatory fields.

m/z value will be calculated automatically based on the provided chemical formula and adduct.

Chemical formulas containing isotopes can be inserted by indicating the isotopologue before the atom letter and by including in square bracket. For example, glucose with 3 heavy carbon (13 Da) can be written as follows:

### [13C]3 C3 H12 O6

Generated target list can be exported/imported as .xlsx.

After completing initial information with the list of files for identification, quantification and relative target table we can now press the Validate button.

### Complete the workflow

				Adducts			
	H+ 🔵	Na+ 🔿		К+ 🔿	NH4+ 🔿	СНЗОН + Н+ 🔾	CH3CN + H+ 🔿
	Н- 🔵	CI- 🔿	Br- 🔿	СНЗСОО- 🔿	HCOO- 🔵	CF3COO- (	) Н2РО4- ()
	ppm value 🔸 5 🕐	RT shift (seconds)	Shift	Peak width (seconds	Peak width		
						Library Name	
1		Х		iroa			
	Select MS/MS libra	ries for analysis	roa		•	Add Lil	brary
		Save and Run			Exi	t	

If all mandatory fields are meet, a new part of the workflow will appear. We can now select the adducts we want to search in positive and negative polarities. At least on adduct for each polarity mast be selected. The correct type of adduct will be searched by matching the chromatographic polarity allowing to analysed data acquired in positive, negative or both ionization mode i.e. switching polarity.

Ppm value need to be specified for the analysis. Ppm values are used for matching all possible chemical formulas within the provided error.

Rt shift need to be specified. This value represent the expected peak shift in retention time within the dataset.

Expected peak width need to be specified only if target compounds are included in the analysis.

Lastly, we can selected a list of fragmentation libraries and add to our analysis. Selected libraries will be used for compound annotation. We can now save the workflow file and start the run. The process will create metafiles required for the analysis within the folder containing the workflow file. We recommend to save the workflow file in an empty folder and do not override existing workflow files as it may lead to analysis mismatches.

During the analysis, it is possible to navigate in the library, but open existing workflows or starting a new analysis is not allowed.



# Open analyzed data

By clicking on the Open button in Home, you can select a workflow file from a previous analysis. The workflow summary will appear.

Annotation and Integration page are now linked to the acquired dataset.

Workflow can not be edited at this stage, but if the workflow was not completed correctly the "Continue" button will appear at the end of the workflow page.

#### Library

By clicking on the Library Icon on the left menu bar, the library page will open.

Two types or libraries are available in this page. Common and user libraries.

Common libraries are shared between all users and cannot be edited or deleted.

User libraries, as the name suggest, are builded by each user and can be edited or deleted at any time. The library manager is shared between all the MSforge software. Therefore, if you own a licence for MSInside, our solution for mass spectrometry imaging analysis, you can use the share library between the two software with identical interface. As the library are stored on MSforge server, updates of the library in any MSforge product will sincronized with all the product available with a valid licence.

Ξ	Crom	imy						<b>o</b> – –				
â				Library name		# Spectra						
	1		hmdb	_experimental_msms	_spec 64917		( I	nspect				
	2		hmdb	_predicted_msms_sp	ectra 1792069		- I	nspect				
षा	3		massb	ank	96065		I	nspect				
	4	Х	iroa		1636			Edit				
				C	reate library							
	iroa											
			Compound name	HMDB ID	Chemical Formula	InChlKey	Collision energy	Polarity				
	103	X	2,5	hmdb0000152	C7H6O4	wxtmdxomehjx	40	-				
	104	X	2,5	hmdb0000152	C7H6O4	wxtmdxomehjx	20	-				
	105	Х	2,5	hmdb0000152	C7H6O4	wxtmdxomehjx	30	-				
	106	X	2-aminophenol		C6H7NO	cdawcloxvubkr	40	+				
	107	Х	2-aminophenol		C6H7NO	cdawcloxvubkr	20	+				
	108	X	2-aminophenol		C6H7NO	cdawcloxvubkr	42	+				
		Add spectra fro	m raw files		Save		Exit					
	_											

#### Create a new library

New user library can be created at any time by clicking on «Create Library» button.



You can now type the name of the new library and click Create.

After few seconds the new library will appear as user library with the spectral count equal to 0.

1     hmdb experimental msms spectra     64917     Inspect       2     hmdb predicted msms spectra     1792069     Inspect       3     X     inspect     126	
2   hmdb predicted msms spectra   1792069   Inspect	
4 X new library 0 Edit	
Create library	

We can now start to populate the new library by clicking the Edit button.

		new library			
Compound name	HMDB ID	Chemical Formula	InChlKey	Collision energy	Polarity
Add spectra from raw files		Save		Exit	

A new area will appear showing all the spectra available in the selected library. In this case none as it was just created.

#### Add spectra to library

By clicking «Add spectra from raw files», we can select a raw data in mzML format that contain the fragmentation spectra of our standards.

After loading the file, a new session will appear below.

			new library			
Co	ompound name	HMDB ID	Chemical Formula	InChlKey	Collision energy	Polarity
			m/z	Rt		
Chemical Formula	+H		<b>▼</b> 5			
Compound name	HMDB		InchlKey		Add to library	Exit

You can now search for your standard by type the chemical formula, adduct, ppm range and name.

HMDB and InchlKey are optional parameters.

After filling up the required fields, the extracted ion chromatogram will appear on the left side.

In the central table, all MS/MS scan matching filter criteria will be displayed reporting the precursor found value and retetion time of the MS/MS event.



By clicking «Select» to one of the spotted MS/MS spectra, it will update both EIC and MS/MS windows. Showing as a red line the MS/MS event compared with the full-scan event (black line) in the EIC, and the MS/MS spectra with found collision energy on the right side of the screen. Both screen help in visualize the quality of the generate MS/MS scan prior selection.



We can now click on «Add to library»,

The compound will now appear on the selected library.

			new library			
	Compound name	HMDB ID	Chemical Formula	InChlKey	Collision energy	Polarity
1 X	glucose 1,6-bisphosphate		C6H14O12P2			
le6 EIC	2		m/z	Rt	Collision Er	nergy 40.0
2.5 -	$\mathbf{v}$	1 Select	338.989654541016	15.48674	- 0000	
2.0 -	$\backslash$	2 Select	338.988830566406	15.844741666667	- 0000	
1.5 -	7	3 Select	338.98876953125	16.244738333333	- 0000	
		4 Select	338.988891601563	16.621238333333	- 0000	
1.0 -					- 0000	
0.5 -					- 0000 - 0 0	
14.5 15.0 15. - · ··	5 16.0				100 150 200	250 300 350 
C6H14O12P2	-Н		<del>~</del> 5	* *	338.98877	
Glucose 1,6-bisphosphate	e HMDI		InchlKey		Add to lib	rary Exit

We can continue to include other MS/MS spectra from the selected files or click «Exit» to go back to the library editing screen.

				new library			
		Compound name	HMDB ID	Chemical Formula	InChlKey	Collision energy	Polarity
1	x	glucose 1,6-bisphosphate		C6H14O12P2			
	Add spectr	a from raw files		Save		Exit	

We can now give a final inspection on the selected library and than click on «Save» to save current changes.

After saving, the number of spectra on the user library will update.

	Library name	# Spectra	
	hmdb experimental msms spectra	64917	Inspect
	hmdb predicted msms spectra	1792069	Inspect
X	iroa	236	Edit
x	new library		Edit
	Create li	brary	

Warning: Common libraries and User libraries are stored on an online server. Poor connection may cause delay in the update process. Connection error (e.g. turning off connection during the process) may cause loss of data. In this case the Library page will change and the logo below will appear indicating missing communication between the machine and the server.



#### Annotation

Direct infusion page allows to evaluate all the annotated compounds in the dataset. If no dataset is open (Home -> Open), or the analysis fail to find any compound this screen will appear.



Otherwise, if the annotation was correctly finished, the annotated compound will be displayed.



In this table, each compound represents a unique identified compound within the dataset. Multiple annotations are already resolved by Crommy e.g. if two peaks may have identical annotations based on mass and fragmentation spectra, the annotation is assigned only to the peak with the best MS2 score and lower MS2 FDR. The assigned compound is removed from the library and the second peak is evaluated against the new library without the already identified compound. Identical annotations may still be present in the dataset if multiple polarities are present.

Ξ	C	rom	my						<b>\$</b>	1 :
-										
บ			1e4	EIC	<u>1</u> e4	lsotopic di	stribution		le4 MS/MS	
<u>N</u>		750 -	$\langle$	<b>`</b>	750 -				200 -	
1	1	500 -			<u>-</u> 500 -				5	
	2	250 -			250 -					
3		0 -			o - L			<u> </u>	0 -	
		7.	5 7.6	7.7 7.8	124	125 126	127 128	129	40 60 80 100 120	
			Index	Name	∧ Formula	Adduct	HMDB	InChl		
		7	631	Valeric acid	C5H10O2	н-	HMDB0000892	NQPDZG		
		8	720	Uric acid	C5H4N4O3	н-	HMDB0000289	LEHOTFF		
		9	4	Trimethylamine N-oxide	C3H9NO	H+	HMDB0000925	UYPYRKY	2.0	ļ
		10	143	Tranexamic Acid	C8H15NO2	H+	HMDB0014447	GYDJEQR	1.5	and its
		11	90	Thiazolidine-4-carboxylic acid	C4H7NO2S	H+	HMDB0258979	DZLNHFI		Into
		12	73	Taurine	C2H7NO3S	H+	HMDB0000251	XOAAWC	0.0	
		13	650	Taurine	C2H7NO3S	H-	HMDB0000251	XOAAWC	126.0	
		14	696	Tartaric acid	C4H6O6	H-	HMDB0000956	FEWJPZIE	125.5	
		15	243	Tacrine	C13H14N2	H+	HMDB0014526	YLJREFD\	Retent: 7.7 7.0 124.5 m/z	
		16	204	TRIETHYL PHOSPHATE	C6H15O4P	H+	HMDB0259191	DQWPFS		
		17	390	Sphingosine	C18H37NO2	H+	HMDB0000252	WWUZIQ	Overlay mode	
	Bv	: MS Fo	orge							1

In this example, we found Taurine twice with index 73 and 650 because this dataset contain data acquired in negative and positive mode and the two peaks represent the same compound at different polarities (H+ and H- adduct) as visible from the overlay of the two peaks in the 3D plot.

In this page, by double click with the left button on the 2d plots is possible to zoom out, while for zoom in we need to click and hold the left button on the x axis of the plot of interest and release the button to zoom in.

For each compound the following columns are present:

Index – This identifier is shared between the dataset, and it is particulary useful for matching peaks with unknown annotation.

Name – Compound name (annotated only otherwise is blank)

Formula – Chemical Formula (annotated only otherwise is blank)

Adduct – Chemical adduct (annotated only otherwise is blank)

HMDB – Human Metabolome ID (annotated only otherwise is blank)

InchlKey - International Chemical Identifier

m/z – mass on charge value

rt - retention time expressed in minutes

charge - evaluated charge

height – maximum peak intensity

area – representative peak area

MS2 type – report the acquisition mode i.e. DDA, DIA or if it is a target compoudn

Ppm – part per million error between the m/z and the chemical formula (annotated only otherwise is blank)

MS2 score – best annotation score against the library relative to annotation

MS2 FDR – false discovery rate estimation based on permutation of the best matching fragmentation spectra in the library

#### Integration

After loading an acquired dataset (Home – Open), by clicking on Integration Icon we can now display the results or our analysis.

Ξ	Crom	my									¢ -
_											
പ		index	name 🔨	formula	inchlkey	HMDB ID	Adduct	Charge	:hrom_switching_`	hrom_switching_{	1e6
N	43	156	N-Acetylthreonine	C6	PED	HMD	H+	1	120191800	121886957	2.0 -
	44	3	N,N	C3	ZMX	HMD	H+	1	263178903	254913412	A.
	45	313	Mepirizole	C11	RHA	HMD	H+	1	10983728	14618382	> <sup>1.5</sup>
	46	745	Mannitol	C6	FBPF	HMD	H-	-1	75577147	75767326	
	47	510	LysoPC(0:0/18:0)	C26	IQG	HMD	H+	1	47134474	51748058	
	48	850	Lumichrome	C12	ZJTJ	HMD	H-	-1	1871916	1933780	0.5 -
	49	890	Linoleic acid	C18	OYH	HMD	H-	-1	11289446	12008059	0.0
	50	304	Leucyl-Valine	C11	MDS	HMD	H+	1	4281098	4365239	7.6 7.7 7.8 7.9 8.0 8.1 8.2 8.3
	51	362	Laurocapram	C18	AXT	HMD	H+	1	7209652	7994300	
	52	82	L-Pipecolic acid	C6	HXE	HMD	H+	1	87966784	92151315	File name
	53	87	L-Isoleucine	C6	AGP	HMD	H+	1	631085553	657478332	chrom_switching_1
	54	127	L-Glutamine	C5	ZDX	HMD	H+		31827597	37301251	2 chrom_switching_2
	55	305	Isoleucyl-Valine	C11	BCX	HMD	H+	1	4281098	4365239	
	56	819	lsokobusone	C14	BSF	HMD	H-	-1	2433618	2566925	
	57	344	Inosine	C10	UGQ	HMD	H+	1	118835509	125861587	
	58	804	Indoxyl sulfate	C8	BXFF	HMD	H-	-1	6538052	6732994	
	59	59	Indole	C8	SIKJ	HMD	H+	1	30214271	30563336	

Together with the summary information for each compound, in the main table we can read the peak area in each sample. To visualize the integrated peak areas, we can select one compound from the main table and the list of chromatograms from the File name table. If we select more than one chromatograms, the peaks are overlayed on the same image space. Double click with left button will zoom out in the chromatogram, while holding the left button and selecting a region on x axis will zoom in. In similar way, by holding the right button and selecting a region on x axis we can reintegrate all the peaks that are displayed. Peak area of the reintegrated peaks will be updated automatically.

By pressing the right button on the central table, we can export the results in excell.

#### Crommy custom tools

The mission of MS Forge is to create software that lightens the workload and increases production in analytical chemistry labs. We are aware that each user may have different needs and requests for their analysis, but may not have the time, financial support, or expertise to develop what it lacks.

For this reason, you can request custom modifications of Crommy to better adapt to your personal needs. From having a different color palette for the GUI to adding a brand-new page with missing functionalities and AI integration, just ask us!

#### info@msforge.it

or

```
https://www.msforge.it/
```

by selecting "Request custom content" on Reason field.

Our team will carefully evaluate your request in terms of feasibility, interest to the general audience, and compliance with general and internal rules.

If we consider the custom modification to be feasible and of general interest, WE WILL DEVELOP IT FOR FREE and include it in the release of the Crommy updates, unless the custom request is expressly requested to be kept private.

Otherwise, if it is doable but not suitable for an Crommy update, we will contact you with an offer regarding the custom request.

Note: Custom requests are accepted only from users owning a regular license (Demo not included).