LC/MS Unknown Identifications Using MSMS Libraries
Part I: Overview of Software and User-Customized Configurations

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- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services*
- *Specialties[1] EI GC-MS, LC-MS/MS, Chemical Ionization,[2] Accurate Mass, Derivatization,[3,4] MS library management, SciFinder[6], Chemspider[6], Surfactant ID,[7] NMR, GC-IR, organic synthesis, matrix ionization effects,[5] etc.*

Eastman Chemical Company, Main Site, Kingsport, TN
50 Manufacturing Sites Worldwide, ~14,500 Employees

>50 Mass Specs Networked
Worldwide

* https://en.wikipedia.org/wiki/Eastman_Chemical_Company

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LC/MS Unknown Identifications Using MSMS Libraries

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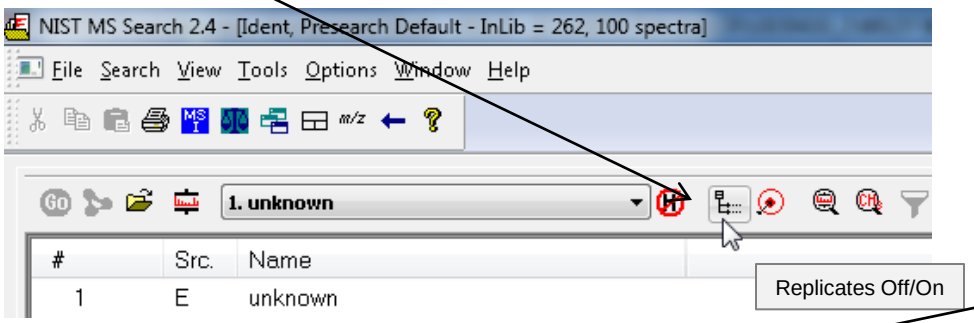
Part VIII: Identification of Unknowns with "Spectraless" Libraries

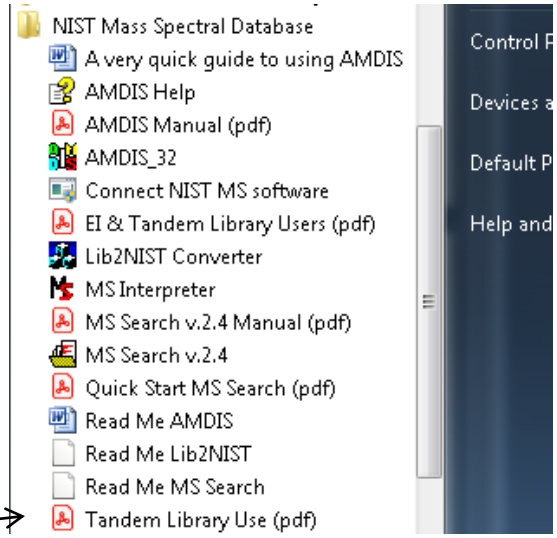
NIST Mass Spectrometry Software and MSMS (Tandem) Libraries

- Free software program for searching and processing MSMS (tandem) libraries
- Aggressive NIST in-house program for obtaining new spectra of purchased compounds
- Supplied with purchased NIST MSMS libraries
- Developed by NIST for curating and searching MSMS libraries
- Import data from variety of instrument manufacturers
- Searches by spectrum, structure, name, CAS No., peaks, MW, MF, etc.
- MS Interpreter[10,17-19] for correlating molecular substructures to fragment ions
- Structure export and import using vendor drawing packages
- Searches other libraries including user, Wiley, MoNA, etc.

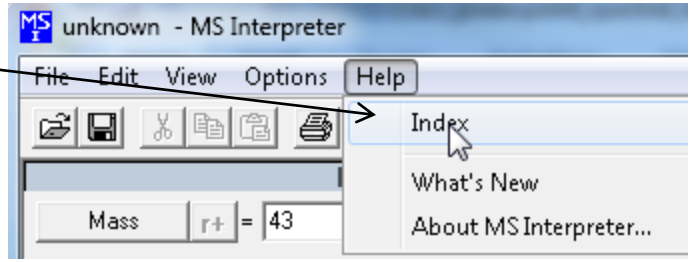
Help Files for NIST Search

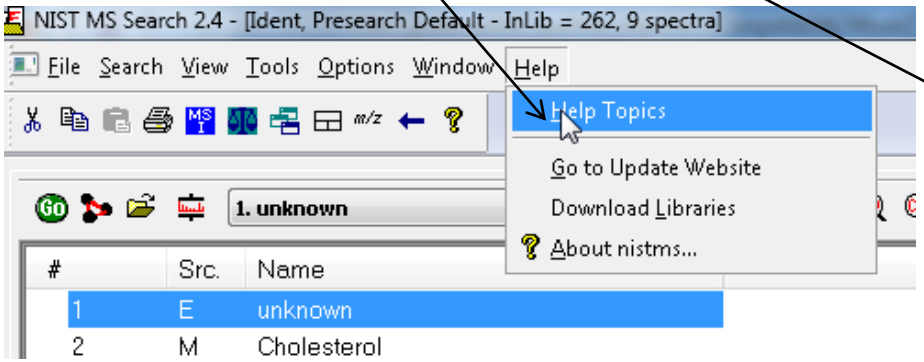
-"Hover" over Program Icon with mouse and function description displayed

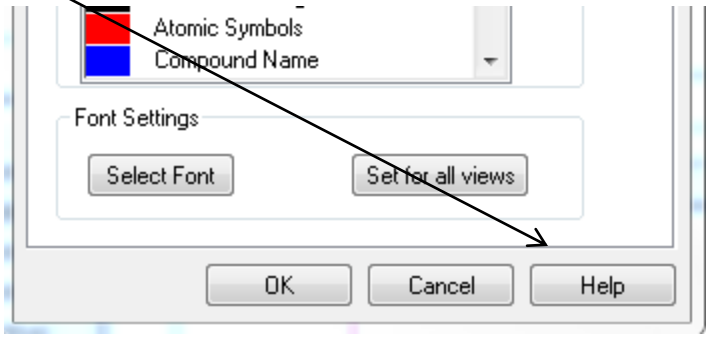




-***Detailed*** documentation for NIST Search[8]
-MS Interpreter included in NIST manual[8]
-Windows Program Group
-NIST Tandem Search ***Quick Start Guide***[9]
-"In program" assistance for both programs
-***Help button*** at corner of all windows



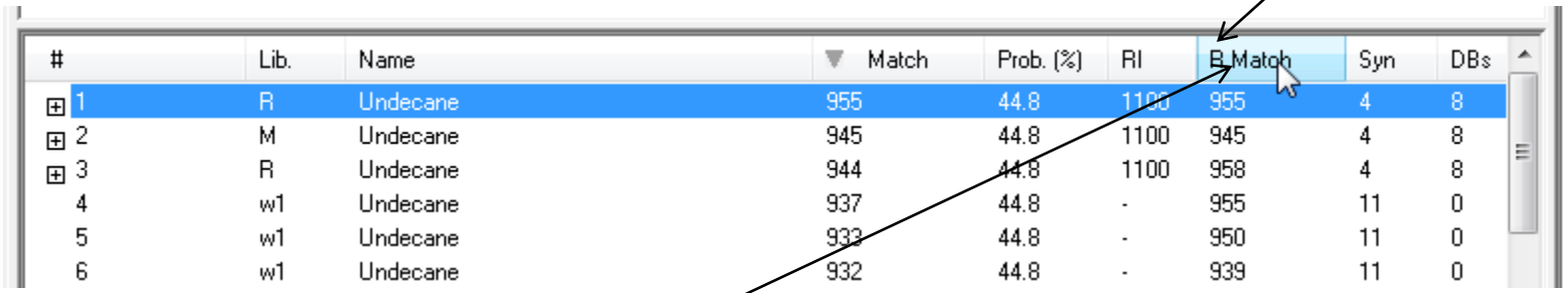




NIST Software in General is "Windows Compliant"

-***left click (LMB)*** to select an item, ***double LMB*** on that item to perform operation
-***right click (RMB)*** in area or item to see operations that can be performed or to change properties of window
-***LMB*** on first item and last item to select group ***while*** holding ***shift key***
-***LMB*** to select/deselect individual items ***while*** holding ***Ctrl button***
-use up and down arrows ***on keyboard*** to step between entries
-some NIST windows such as librarian have no delete button to delete ions, ***must*** use delete key on keyboard!
-***control a*** (select all), ***control x*** (delete selected), ***control c*** (copy); ***control v*** (paste)
-***control k*** copies entries into windows in tab-separated text format, e.g., ***paste*** into Excel
-F1 MS Search help
-F9 send spectrum to MS Interpreter

Tip 3: ***LMB*** and **drag** to rearrange order of column headers



#	Lib.	Name	Match	Prob. (%)	RI	R Match	Syn	DBs
1	R	Undecane	955	44.8	1100	955	4	8
2	M	Undecane	945	44.8	1100	945	4	8
3	R	Undecane	944	44.8	1100	958	4	8
4	w1	Undecane	937	44.8	-	955	11	0
5	w1	Undecane	933	44.8	-	950	11	0
6	w1	Undecane	932	44.8	-	939	11	0

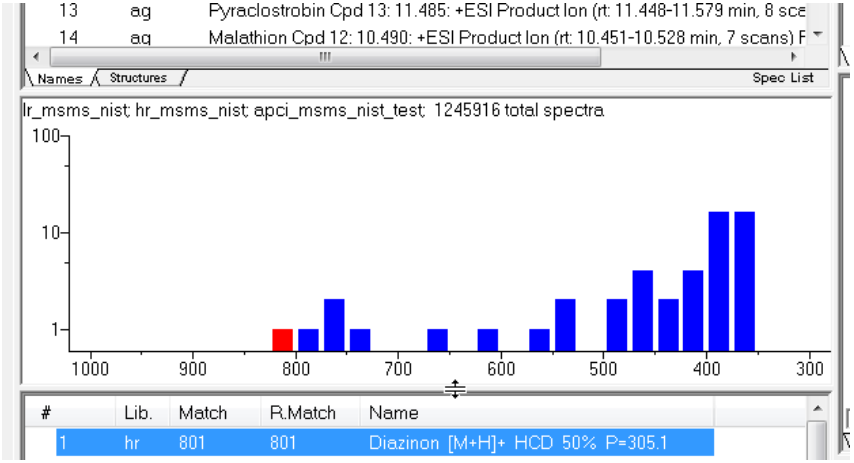
-***LMB*** on column of interest
-Can sort in lower value first or higher

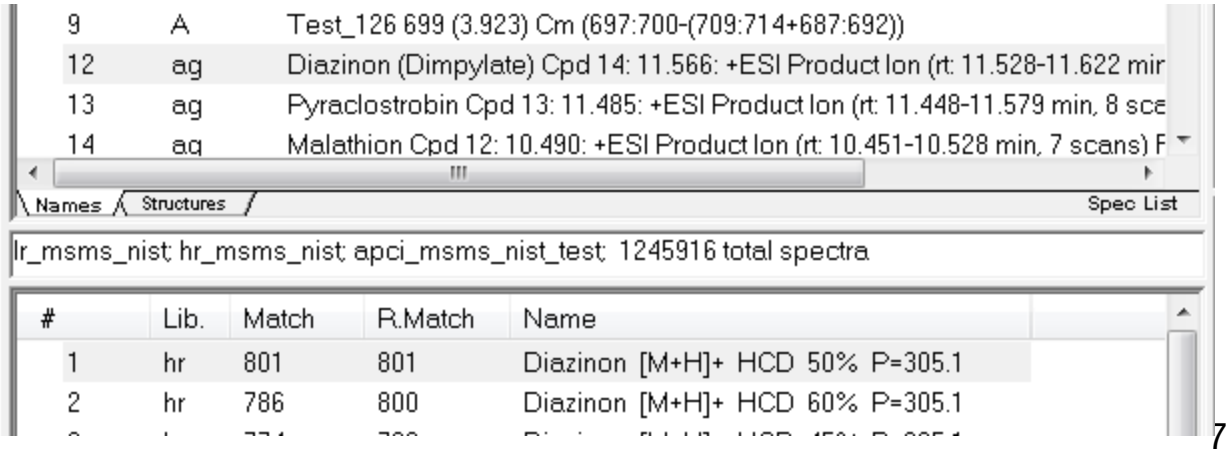
Tip 1: When reviewing search results, use up and down arrows on keyboard to quickly step through results!

Tip 2: When viewing structures in MS Interpreter, use left and right arrows on keyboard to quickly review results!

Customizing the Windows

- place cursor over any bar between (***top or side***) windows and then ***LMB*** and drag to change the size of a window or make window so small it essentially disappears
- *E.g.,* I prefer to minimize the middle window bar graph and only display libraries searched

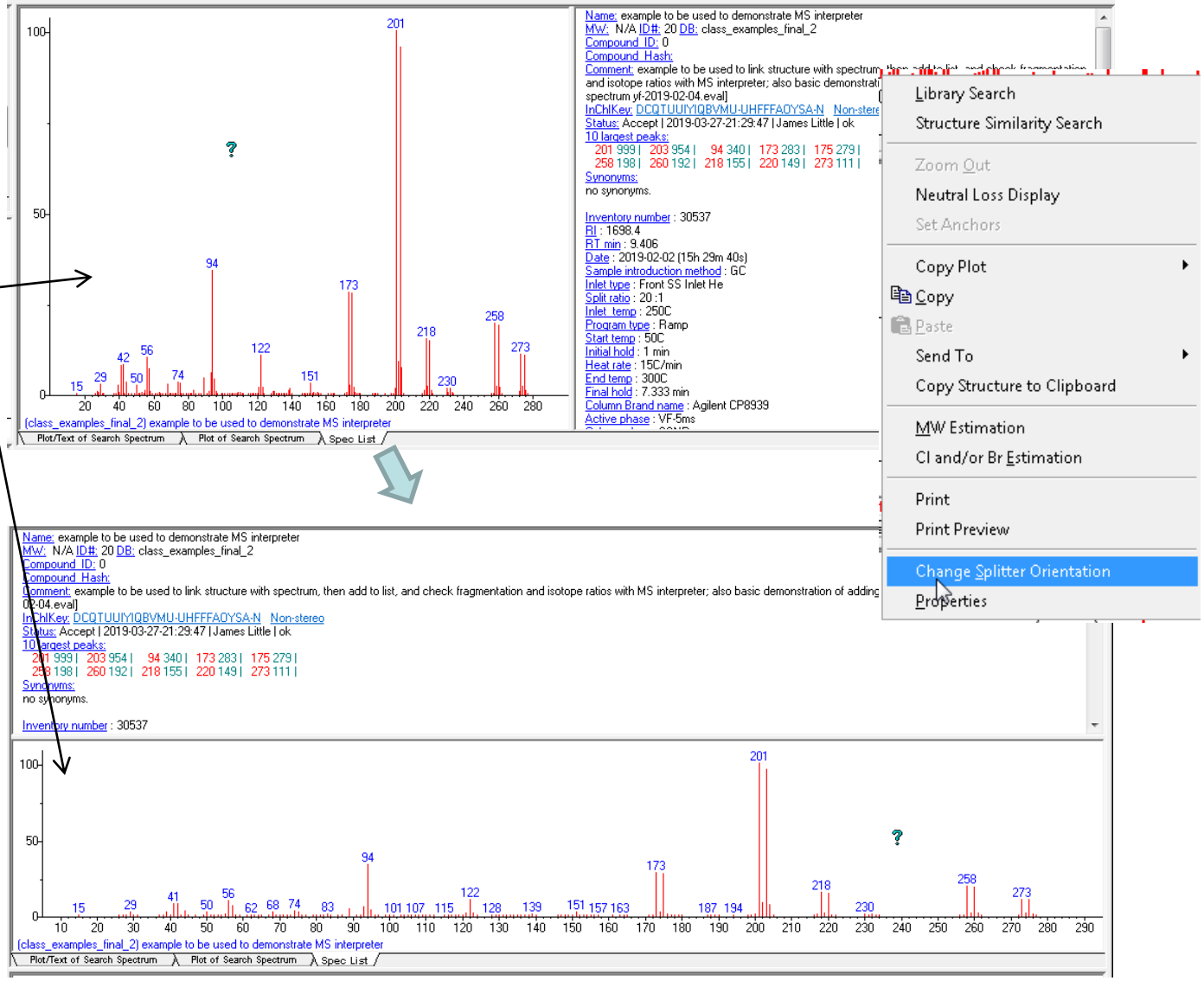




Customizing the Windows (continued)

-RMB in display windows then ***LMB*** to “Change Splitter Orientation”

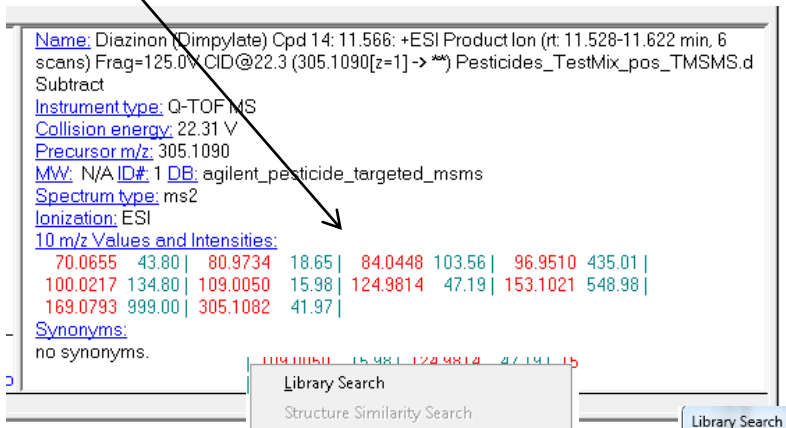
Tip: ***All*** mass spectra in windows can be ***“Boxed”*** to ***expand*** by ***LMB*** and dragging to expand/enlarge; ***restore*** to original by ***RMB*** in spectrum region and selecting ***“Zoom Out”***



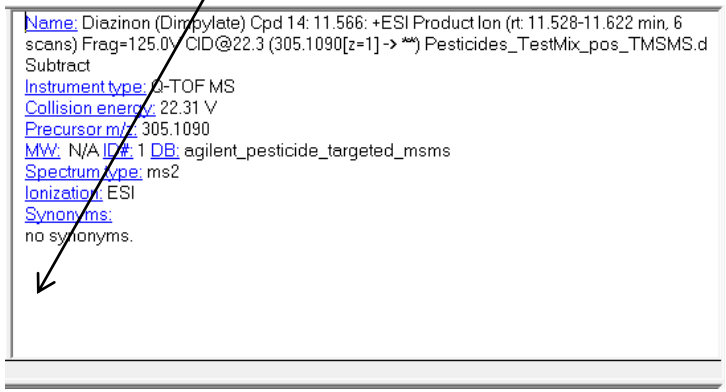
Customizing the Windows (continued)

- Removing *unnecessary items* in displays
- *E.g.* Removing *m/z* intensity list

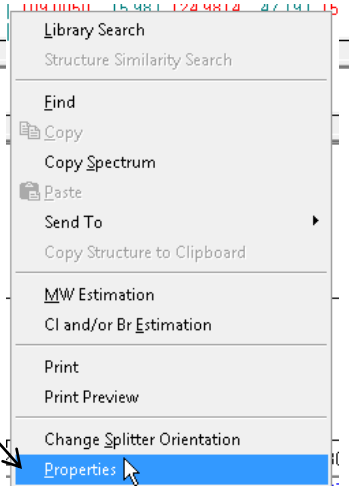
1) **RMB** anywhere in window

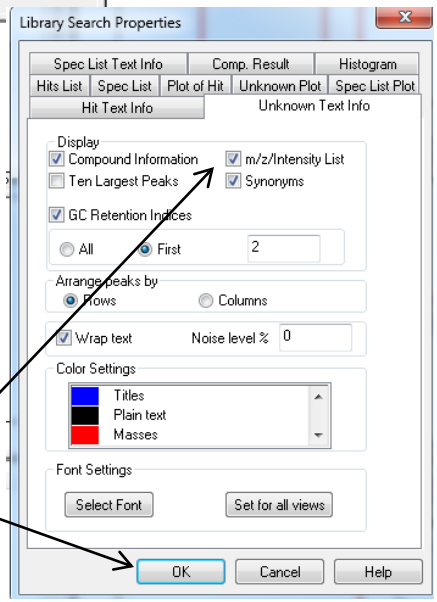


4) *m/z* intensity pairs removed from display



2) **LMB** properties



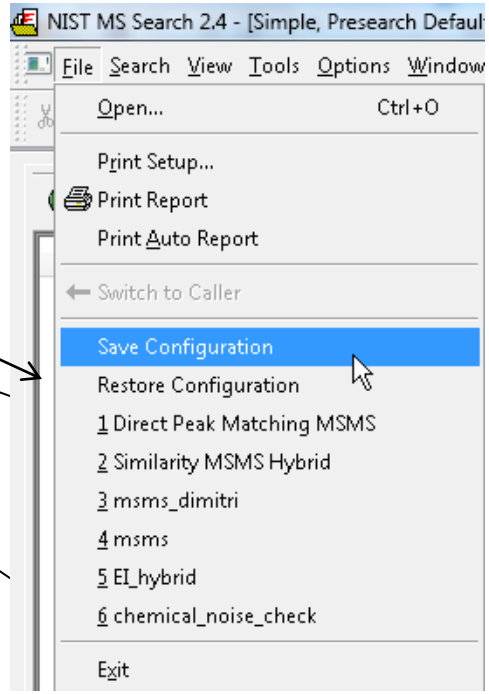


3) Deselect “m/z intensity list” then **LMB** OK

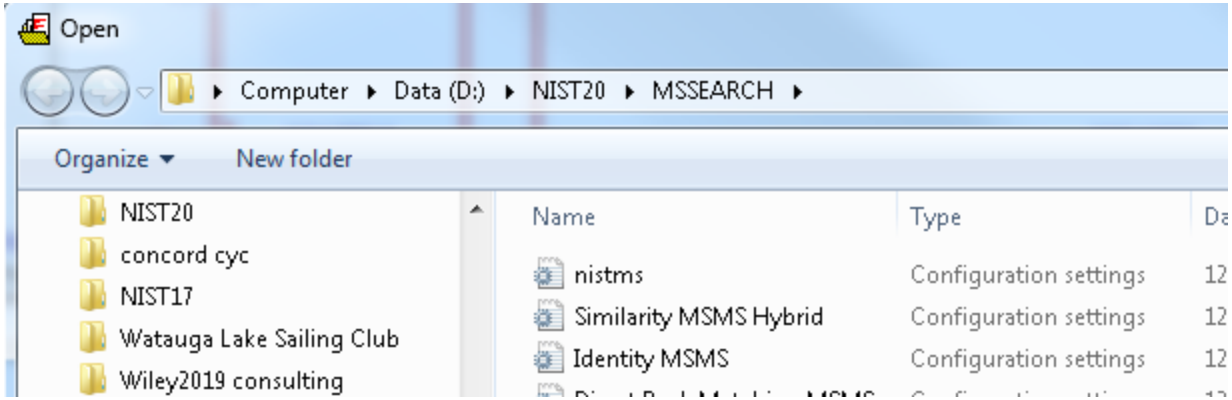
Saving and Restoring User Configurations

-Restore and Save user Configurations

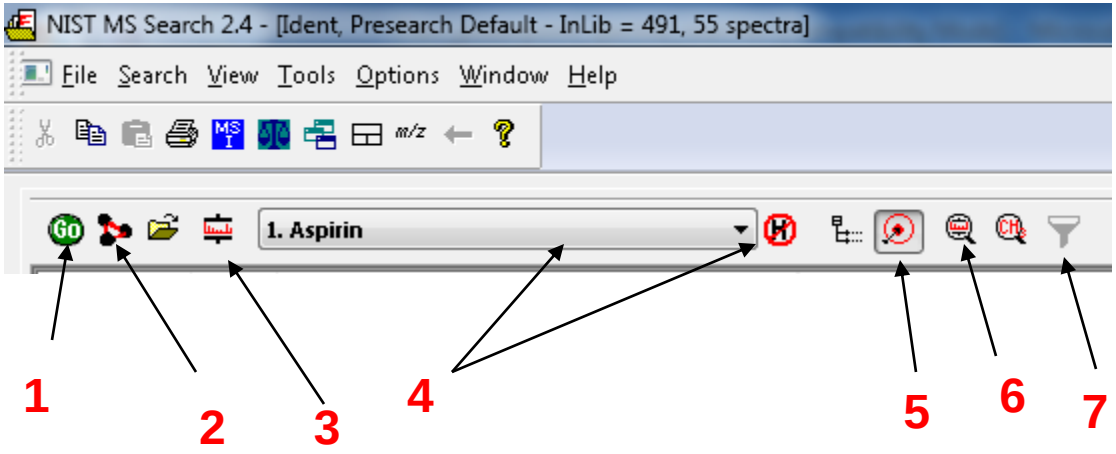
- Most used listed (1) or select from a file listing (2)



2



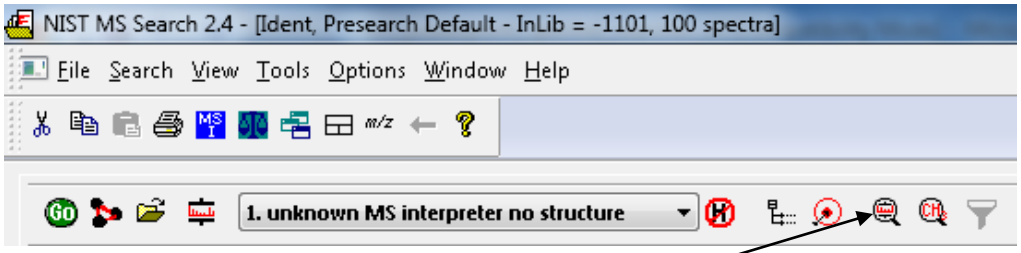
Main Functions on Toolbar

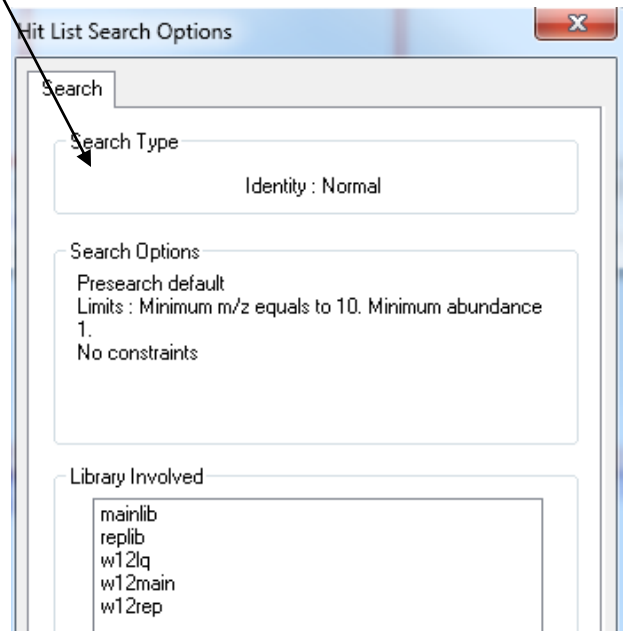


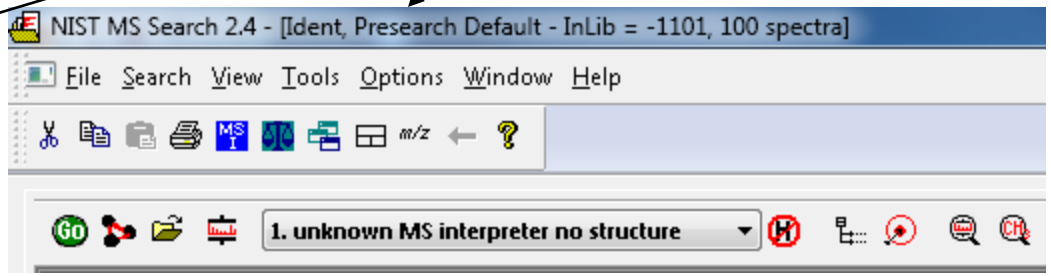
1. ***LMB*** to start search or ***double LMB*** on entry in spec list window
2. ***LMB*** to do structure search
3. ***Critical*** user settings for structure and spectra searches
4. Search results stored and ability to clear list
5. Only show best hit of entry with same CAS number, minimizes looking at redundant entries in search window
6. View search options used on *last* search performed
7. Filter settings to remove spectra from search list by type of ion, polarity, type of instrument, etc.

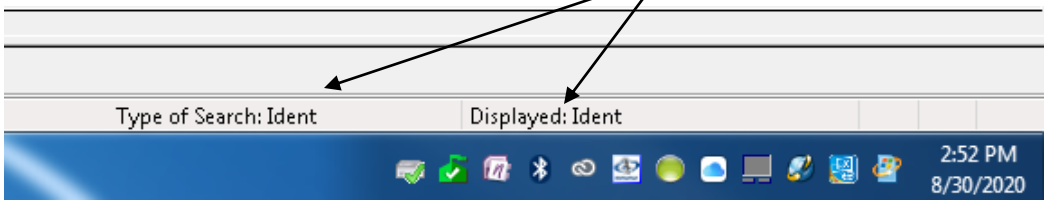
View of Search Options *Employed*

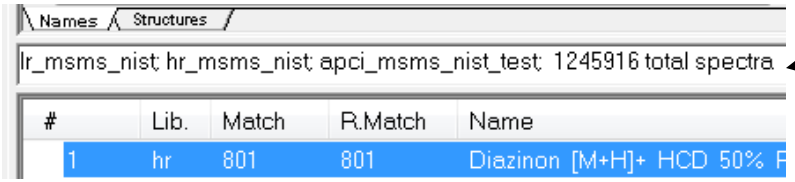
1. ***LMB*** the “View Hit List Search Options”
2. See details of the last search performed
3. Also, an abbreviated description of the last search noted at ***top*** of NIST search window
4. Furthermore, at the ***bottom*** of the screen, *respectively* displayed, are the types of searches that ***will*** be performed and the type of results ***currently*** displayed
5. List of libraries searched with total # spectra



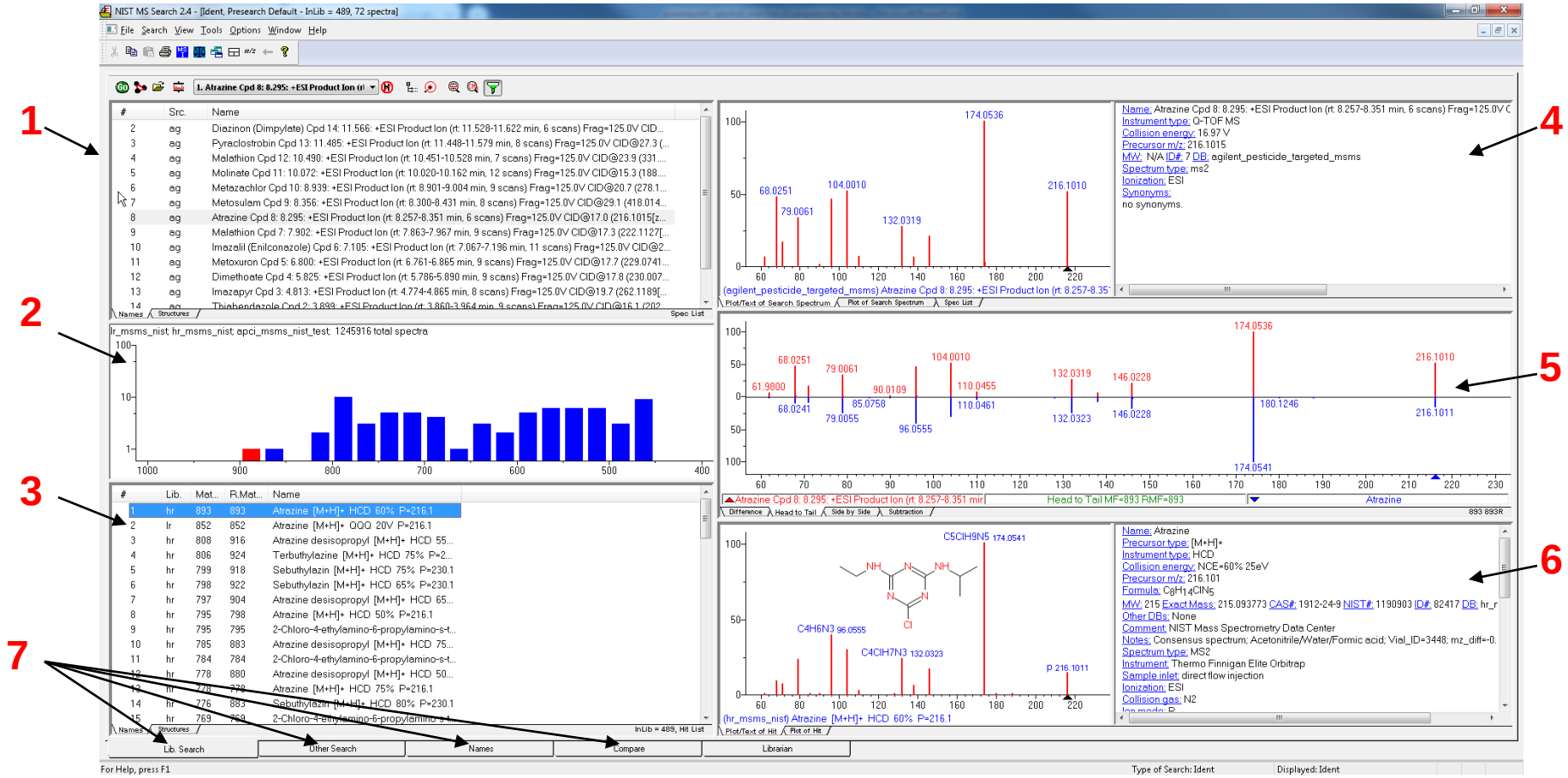






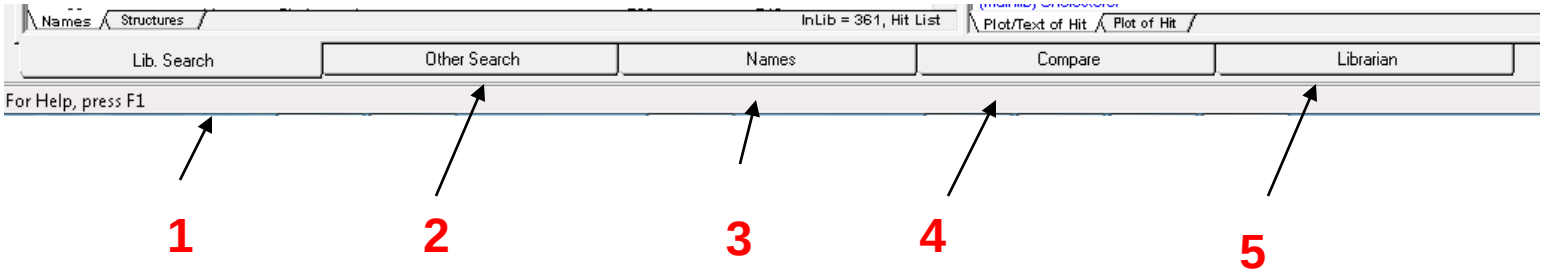


Main Window in Lib Search View



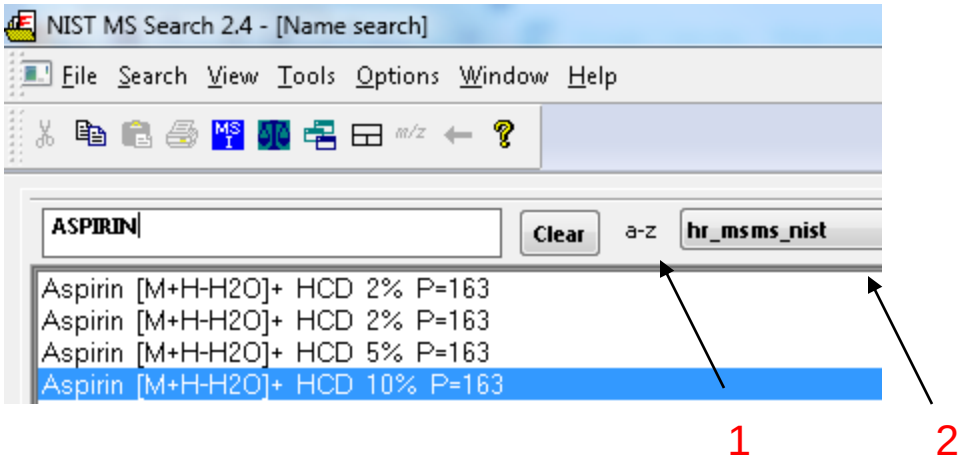
1. Spec list window for import of spectra and structures from other programs
2. Histogram, Statistics on search
3. Hits list, step through by ***LMB*** then ***up*** and ***down arrows on keyboard***
4. Unknown spectra and info
5. Comparison of unknown to selected hit result, ***many different display options*** with tabs at bottom left of window
6. Spectrum of hit and other associated information
7. Accessing other windows and associated functions ***Other Search***, ***Names***. ***Compare***, ***Librarian***

Tabs for Other Functions Accessed at *Bottom* of Main Library Page (Detailed Discussions in *Future* Sessions for Tabs *4* and *5*)



1. ***Lib Search***-main window for searching spectra and structures
2. ***Other Search***-search by CAS, MW, ID no., partial name, MF, etc.
3. ***Names***-search by name, *e.g.* see *aspirin on next slide*
4. ***Compare***-Window used to compare spectra, also can display best hits from search
5. ***Librarian***-window used to edit spectra, correlate spectra with structure, create user libraries, type in spectra manually, etc.

Names Search Tab



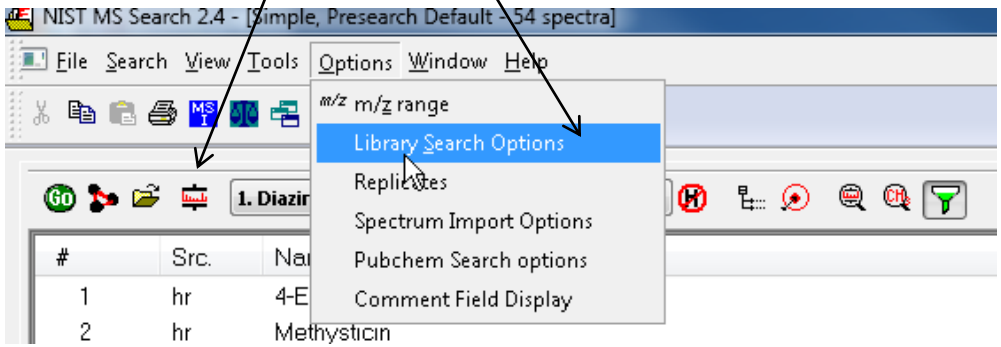
1. *Toggle: letters only or letters/numbers*
2. *Search **one library** at a time*

Setting Up Search Parameters for MSMS Searches

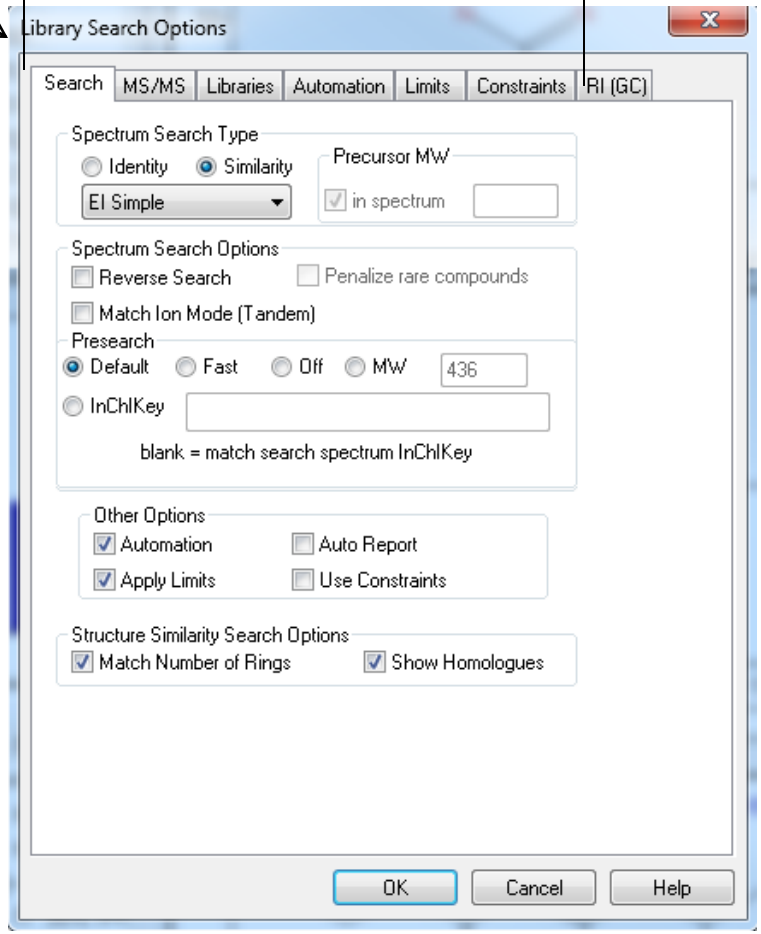
Critical Step

-Critical part of process is user setting up search options
-Use “Library Search Options”
-Values selected in *all* “tabs” on submenu *except* RI (GC)
-Actual values discussed in *Part II* of the series

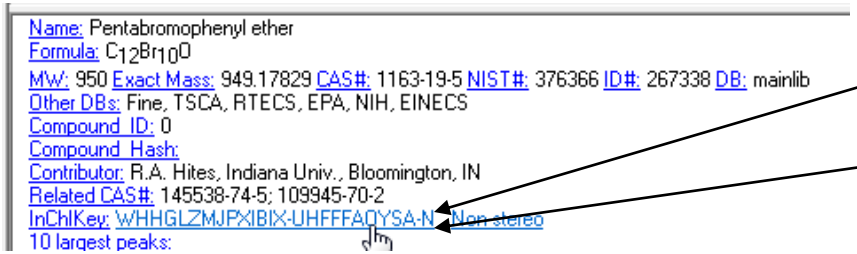
Two ways to access, *icon* on toolbar or *menu*



Values entered in ***all*** Tabs ***except*** RI (GC)

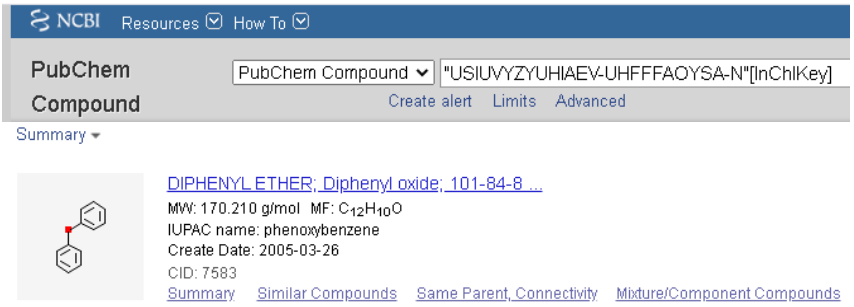


InChIKey Field Link to PubChem on Web

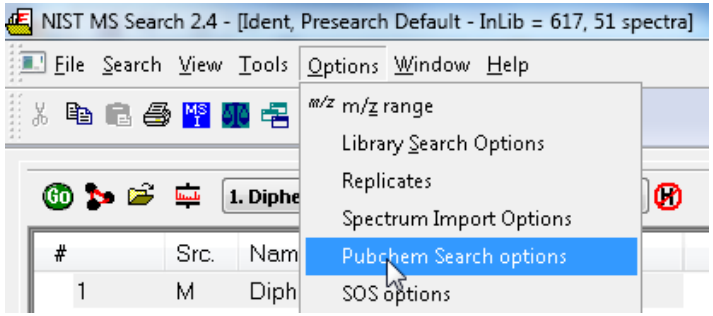


-InChIKey is ASCII “hashed” representation of structure* and is searchable

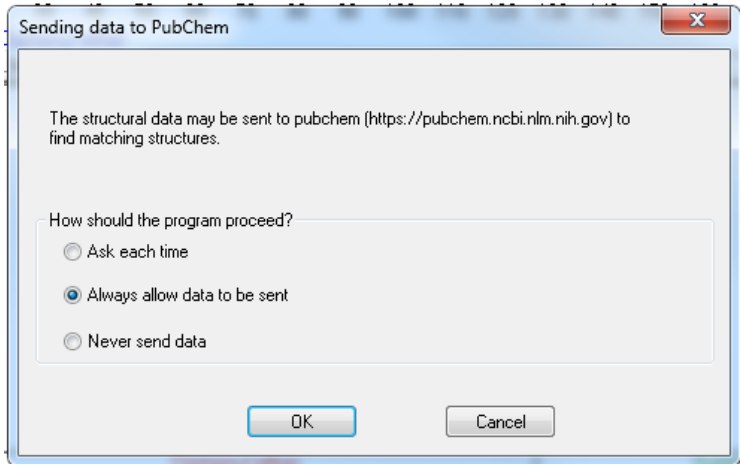
-Double ***LMB*** on ***any*** InChIKey in NIST software and you will be taken to PubChem Web Page on internet



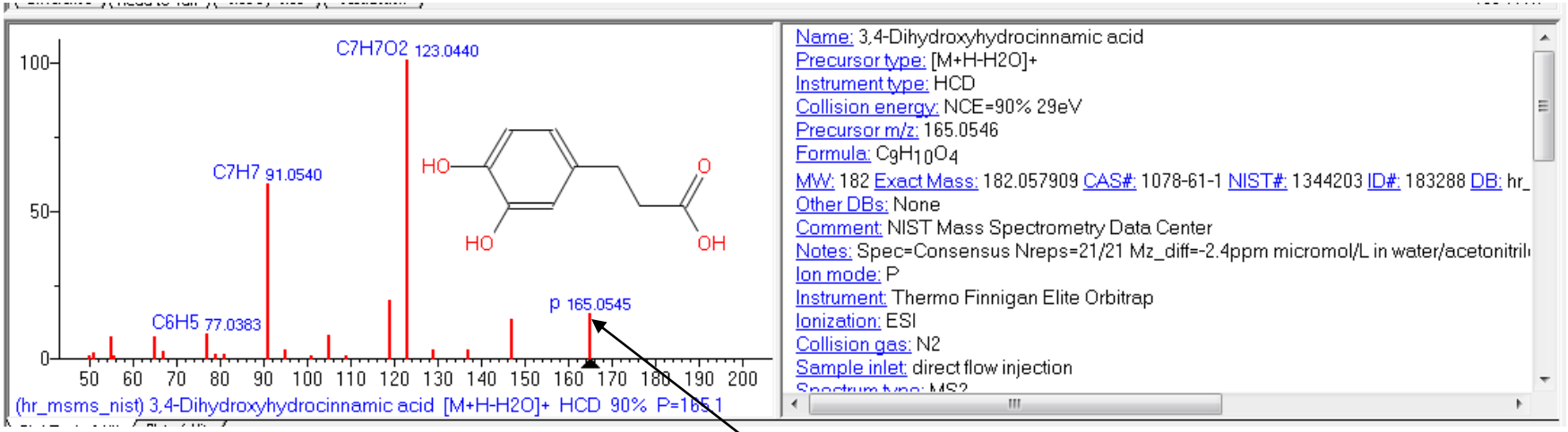
Selecting PubChem Options:

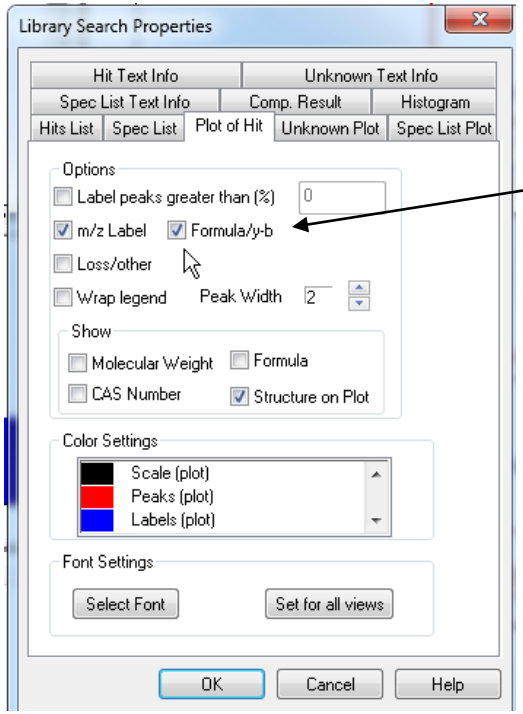


* https://en.wikipedia.org/wiki/International_Chemical_Identifier



Molecular Formulae Can be Shown in High Resolutions (HR) MSMS



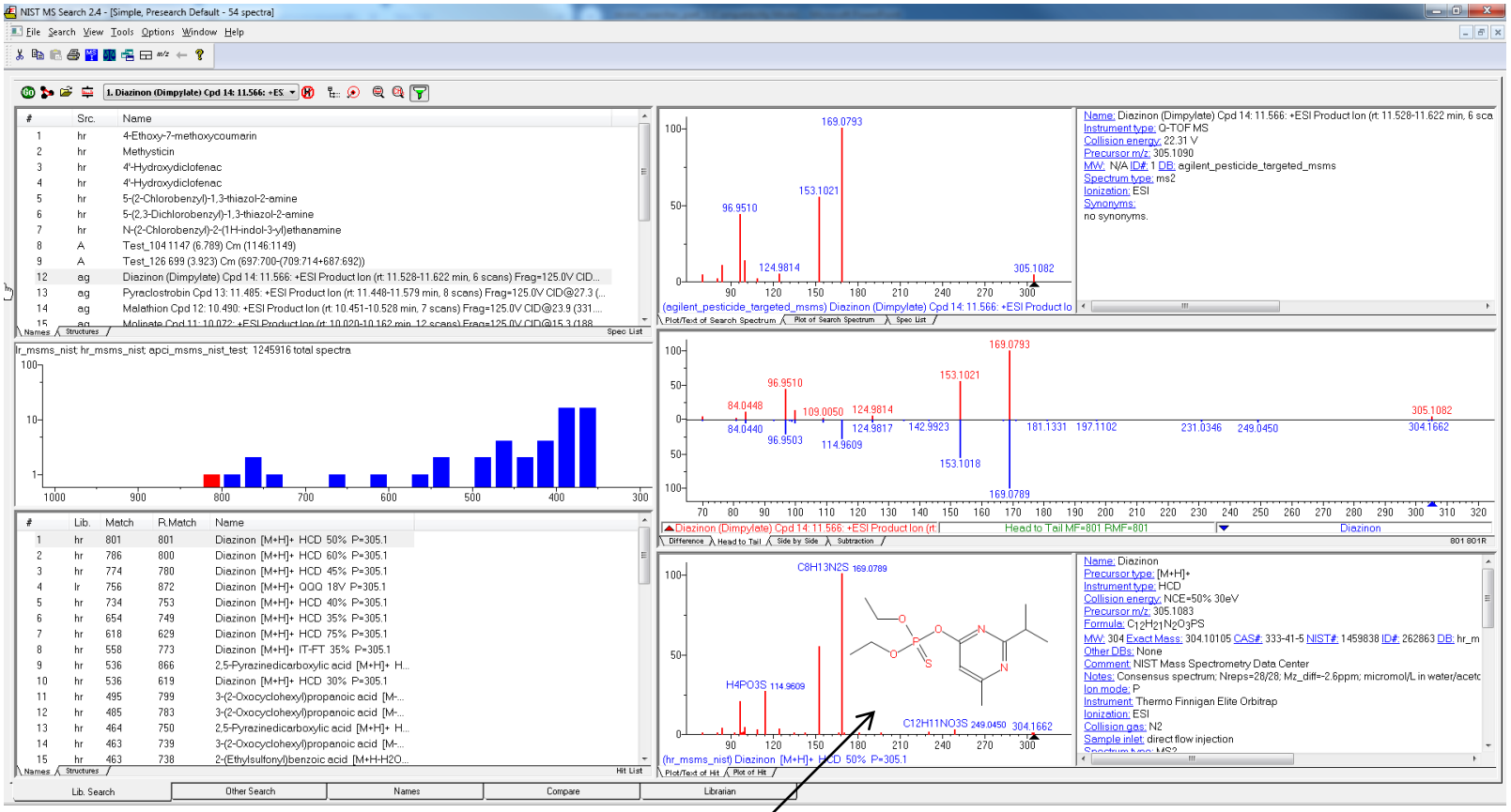


Select to show molecular formulae
“p” is for precursor

Live Demo on YouTube

Part I: Overview of Software and User Customized Configurations

-Live demo summarizes basic functions used in MSMS searches and saving configurations



Tip: ***All*** mass spectra in windows can be ***"Boxed"*** to ***expand*** by ***LMB*** and dragging to expand/enlarge; ***restore*** to original by ***RMB*** in spectrum region and selecting ***"Zoom Out"***

Presentation References (*Internet Links*)

1. *James Little Mass Spectral Resource Website*
2. *Chemical Ionization for MW Determination*
3. *Trimethylsilyl Derivatives for GC-MS*
4. *Methyl Ester Derivatives for GC-MS*
5. *Lipid Matrix Ionization Effects in LC-MS*
6. *SciFinder/ChemSpider and Accurate Mass LC-MS Data for Unknown ID's*
7. *Surfactant Identification*
8. *NIST Search Software Detailed Manual*
9. *NIST Tandem Quick Start Guide*

Acknowledgements

- Stephen Stein (NIST)
- Dmitri Tchekhovskoi (NIST)
- David Sparkman (NIST Contractor)