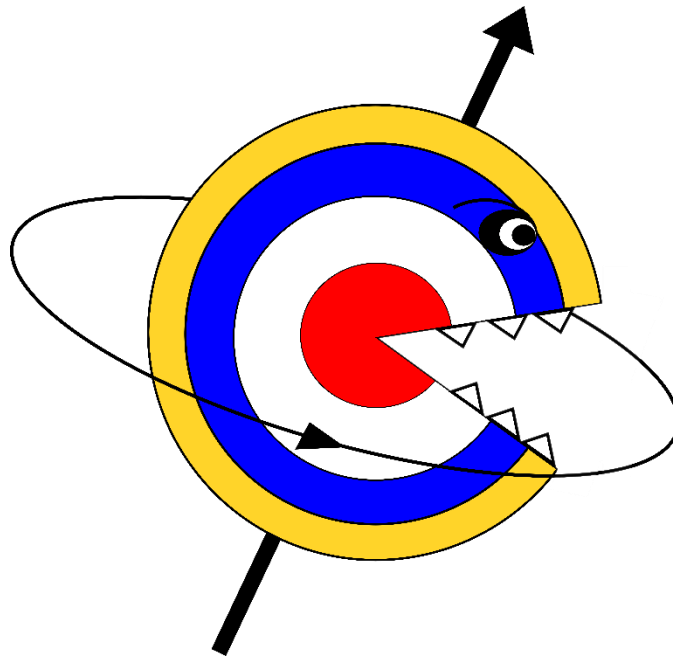


MADByTE Quick Start Tutorial

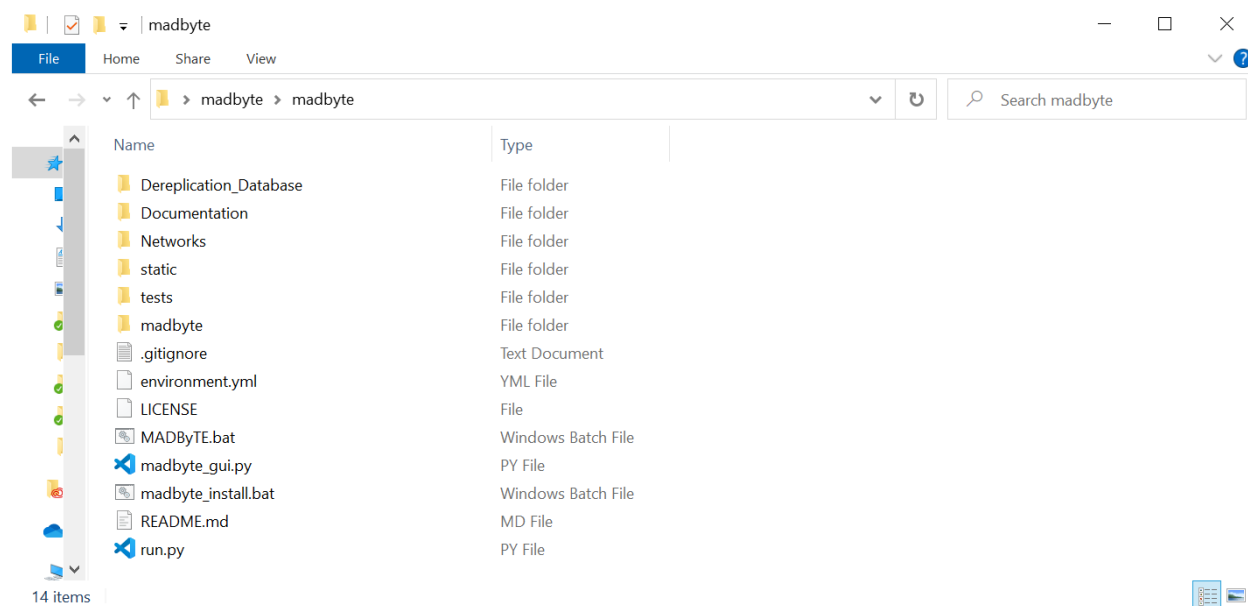


Step 1: Gather Data

MADByTE requires processed and peak picked NMR data to perform analysis. For this example, these data can be found and downloaded at <https://zenodo.org/record/3825107>. To use your own data, please see the User Manual for detailed requirements.

Step 2: Launch MADByTE

Launching MADByTE can be done by opening a terminal window in the directory where the MADByTE code base is located. That directory will look like this:



In the terminal, navigate to this directory.

- One method (although not the only one) is to open anaconda prompt and change directory by using 'cd'. An example is shown below.

```
(base) C:\Users\Jmegan>cd desktop  
(base) C:\Users\Jmegan\Desktop>cd madbyte  
(base) C:\Users\Jmegan\Desktop\madbyte>cd madbyte  
(base) C:\Users\Jmegan\Desktop\madbyte\madbyte>
```

Then, activate the madbyte environment by using the command 'conda activate madbyte'. This will ensure we are using the anaconda environment set up by the install script. Then, launch MADByTE by using the command 'python madbyte_gui.py'.

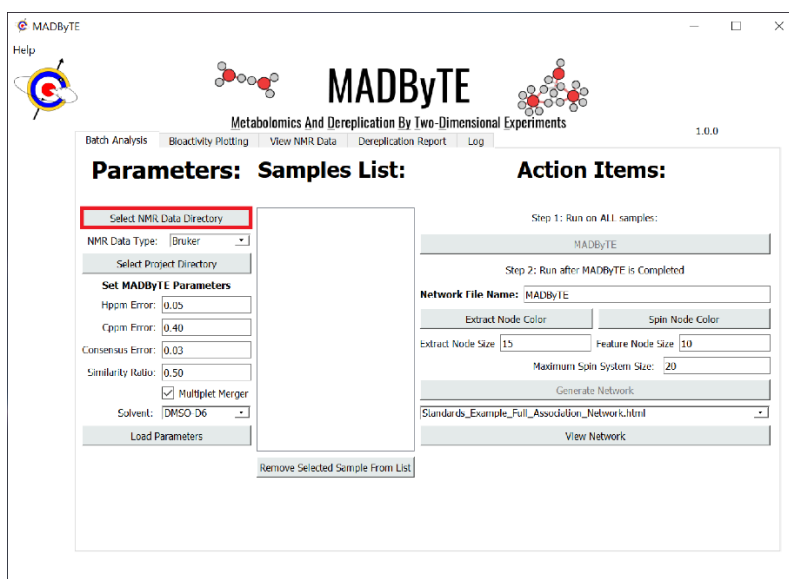
```
(base) C:\Users\Jmegan\Desktop\madbyte\madbyte> conda activate madbyte

(madbyte) C:\Users\Jmegan\Desktop\madbyte\madbyte>python madbyte_gui.py
MADByTE is loading...
Multithreading with maximum 8 threads
```

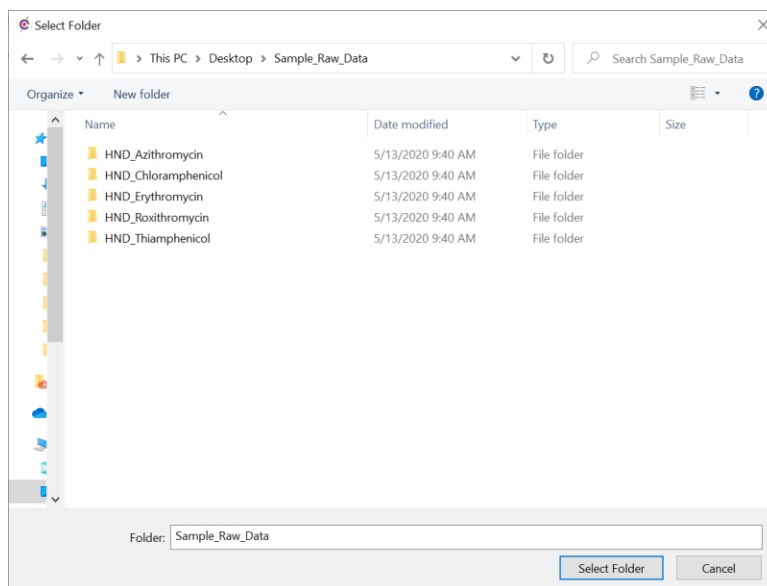
On windows, you can launch MADByTE by using the “MADByTE.bat” file. This script will check to ensure anaconda and the dependencies are properly installed before launching.

Step 3: Set up MADByTE Parameters

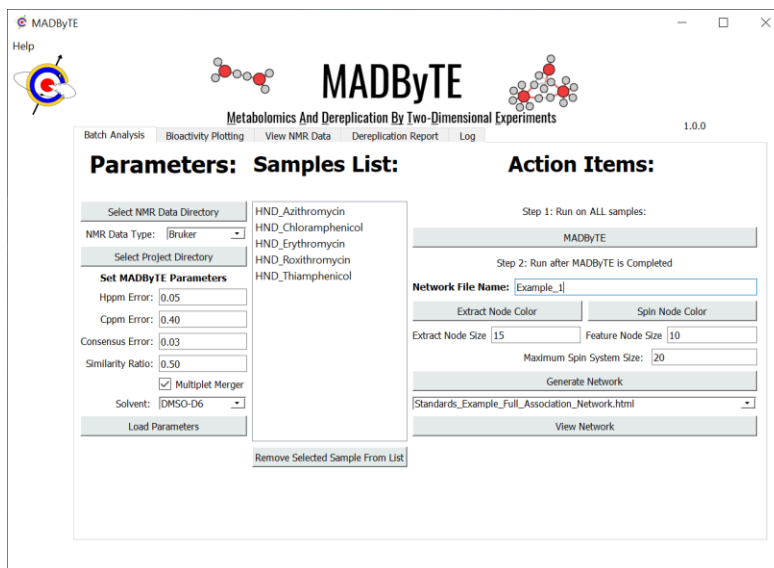
Users are required to select an NMR directory and a project directory before MADByTE can be run. To do this:



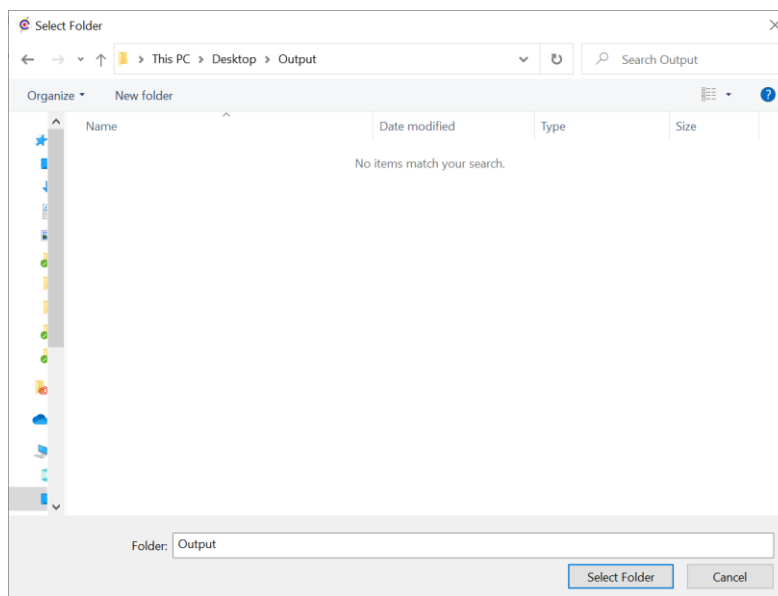
This will open a file dialog which allows you to navigate to the directory where your NMR data is stored.



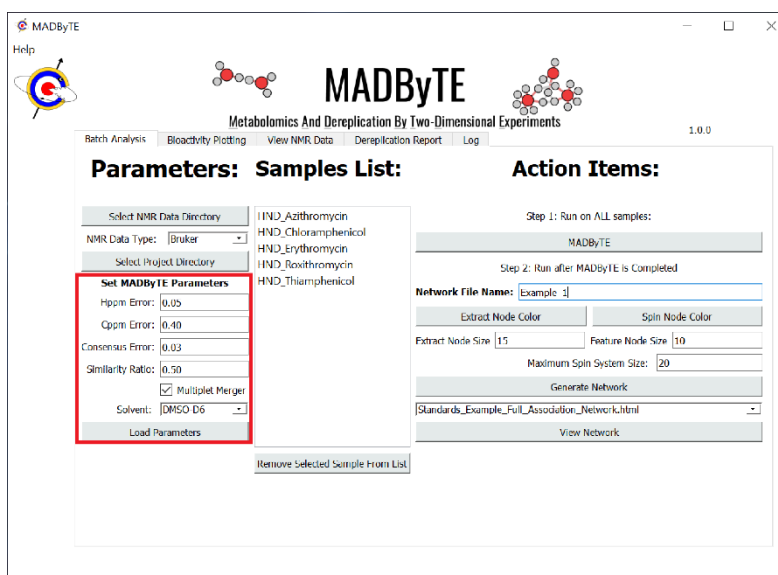
Here, you simply want to select the directory containing your samples, not each sample file folder. After selecting the folder, you will see the samples list populate with the samples from the directory.



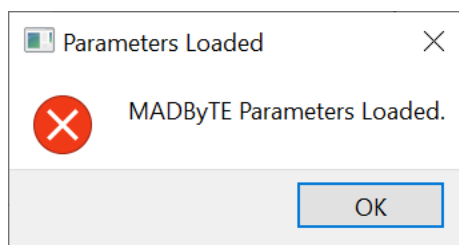
Repeat this step for the Project Directory – This is where the output files will be stored. There will not be any change to the Main GUI when a project directory is selected.



Next, the user can adjust the settings in the space below. This allows for users to adjust the Hppm Error, Cppm Error, the Consensus Error, and Similarity Ratios. Detailed information on what each of these does to the processing can be found in the main user manual.

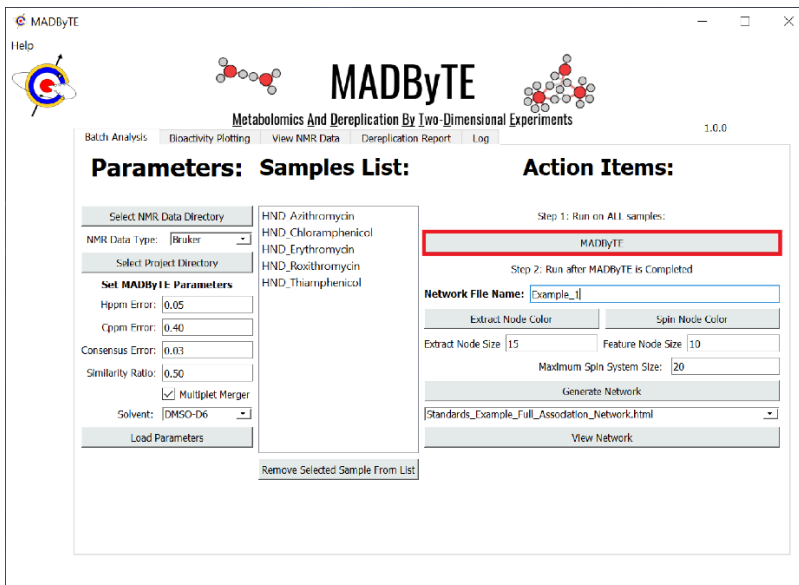


After adjusting these parameters, select “Load Parameters” and a message will display showing the parameters have been loaded.

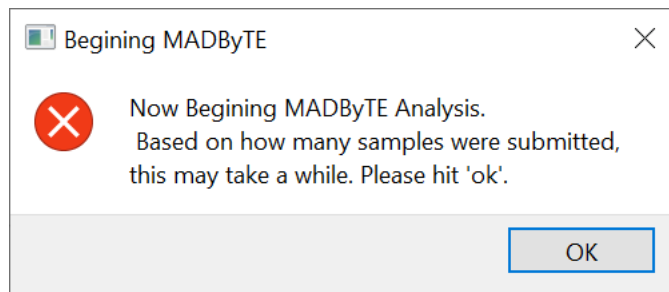


Step 4: Run MADByTE

Once parameters have been set, run MADByTE analysis using the MADByTE button.



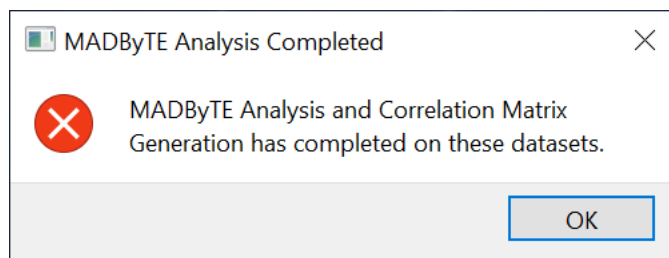
Users will get the following message to show that MADByTE analysis has begun. Select OK to begin processing.



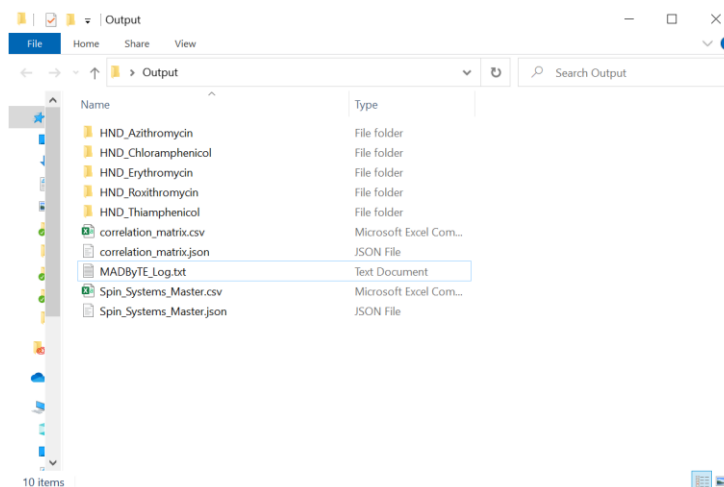
During this process, detailed information will be displayed in the terminal window. This information is also stored in the log file.

```
C:\WINDOWS\system32\cmd.exe
2020-11-24 08:52:57,293 - MADByTE: DEBUG : HND_Roxithromycin : Found 1 HSQC protons to match TOCSY proton 3.622
2020-11-24 08:52:57,293 - MADByTE: DEBUG : HND_Erythromycin : 1.069 removed from spin network
2020-11-24 08:52:57,294 - MADByTE: DEBUG : HND_Roxithromycin : Found 1 HSQC protons to match TOCSY proton 3.896
2020-11-24 08:52:57,295 - MADByTE: DEBUG : HND_Erythromycin : 4.347 removed from spin network
2020-11-24 08:52:57,296 - MADByTE: DEBUG : HND_Erythromycin : 1.168 removed from spin network
2020-11-24 08:52:57,296 - MADByTE: INFO : HND_Roxithromycin finished processing
2020-11-24 08:52:57,296 - MADByTE: DEBUG : HND_Erythromycin : 4.056 removed from spin network
2020-11-24 08:52:57,297 - MADByTE: DEBUG : HND_Erythromycin : 2.27 removed from spin network
2020-11-24 08:52:57,298 - MADByTE: DEBUG : HND_Erythromycin : 4.719 removed from spin network
2020-11-24 08:52:57,298 - MADByTE: DEBUG : HND_Erythromycin : 2.896 removed from spin network
2020-11-24 08:52:57,298 - MADByTE: DEBUG : HND_Erythromycin : 3.06 removed from spin network
2020-11-24 08:52:57,299 - MADByTE: DEBUG : HND_Erythromycin : 3.74 removed from spin network
2020-11-24 08:52:57,299 - MADByTE: INFO : HND_Erythromycin : Final spin network has 23 nodes and 39 edges
2020-11-24 08:52:57,302 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 0.761
2020-11-24 08:52:57,305 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.378
2020-11-24 08:52:57,306 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 5.11
2020-11-24 08:52:57,307 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.796
2020-11-24 08:52:57,309 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.018
2020-11-24 08:52:57,310 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 3.48
2020-11-24 08:52:57,311 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.895
2020-11-24 08:52:57,312 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 4.034
2020-11-24 08:52:57,313 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 4.693
2020-11-24 08:52:57,315 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.074
2020-11-24 08:52:57,316 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 2.463
2020-11-24 08:52:57,318 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 3.62
2020-11-24 08:52:57,320 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 3.037
2020-11-24 08:52:57,321 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 4.368
2020-11-24 08:52:57,323 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.606
2020-11-24 08:52:57,324 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.106
2020-11-24 08:52:57,325 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 2.769
2020-11-24 08:52:57,326 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 4.014
2020-11-24 08:52:57,328 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.526
2020-11-24 08:52:57,329 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 2.294
2020-11-24 08:52:57,330 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 4.74
2020-11-24 08:52:57,331 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 1.714
2020-11-24 08:52:57,332 - MADByTE: DEBUG : HND_Erythromycin : Found 1 HSQC protons to match TOCSY proton 2.86
2020-11-24 08:52:57,339 - MADByTE: INFO : HND_Erythromycin finished processing
2020-11-24 08:52:57,467 - MADByTE: INFO : Generating correlation network...
100% | 361/361 [00:02:00:00, 131.27it/s]
```

Once MADByTE has completed analysis, the following message will be displayed:

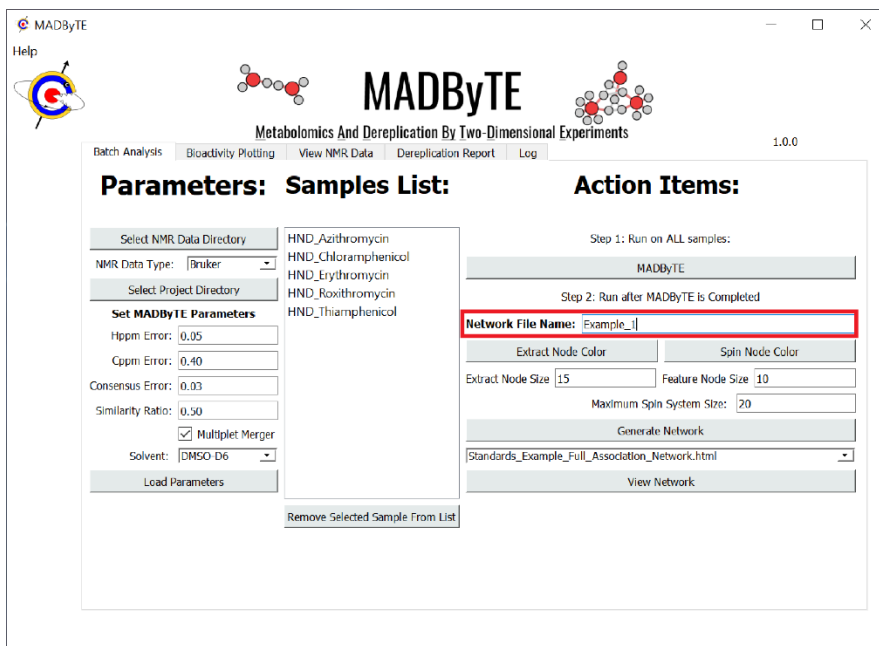


Once this has completed, the Project Directory will now have a suite of new files in it. These are used to construct the MADByTE network.



Step 5: Generate Networks

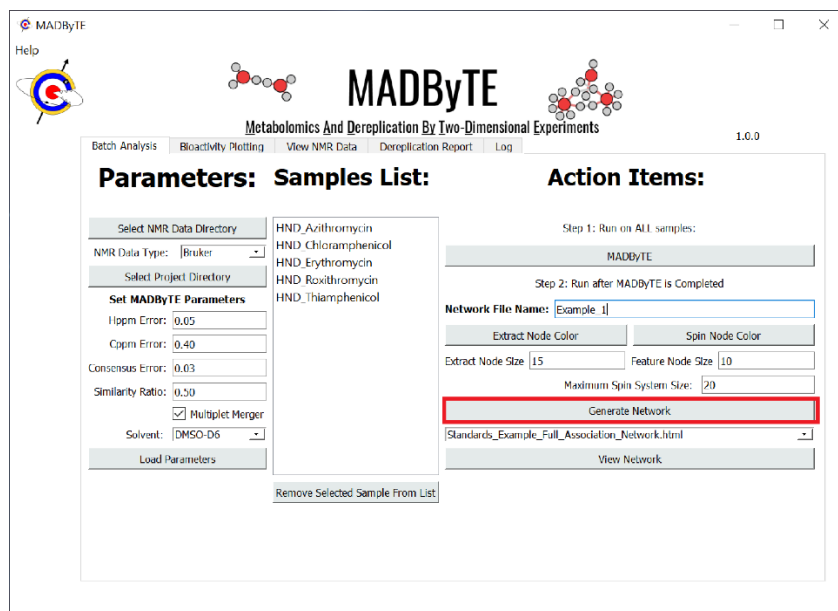
Once MADByTE has completed, the network generation will take the spin system information and the correlation matrix information and construct a network. To give the network a customized name, fill in the “Network File Name” field.



The screenshot shows the MADByTE software interface. The title bar reads "MADByTE" and the subtitle is "Metabolomics And Dereplication By Two-Dimensional Experiments". The version number "1.0.0" is in the top right. The interface is divided into three main sections: "Parameters:", "Samples List:", and "Action Items:". The "Parameters:" section includes fields for "Select NMR Data Directory", "NMR Data Type" (set to "Bruker"), "Select Project Directory", "Set MADByTE Parameters" (with "Hppm Error" at 0.05, "Cpdm Error" at 0.40, "Consensus Error" at 0.03, and "Similarity Ratio" at 0.50), a checked "Multiplet Merger" checkbox, and a "Solvent" dropdown set to "DMSO-D6". The "Samples List:" section contains a list of samples: "HND_Azithromycin", "HND_Chloramphenicol", "HND_Erythromycin", "HND_Roxithromycin", and "HND_Thiamphenicol". The "Action Items:" section shows two steps: "Step 1: Run on ALL samples:" with a "MADByTE" button, and "Step 2: Run after MADByTE is Completed" with a "Network File Name" field (containing "Example_1") highlighted by a red box, and buttons for "Extract Node Color", "Spin Node Color", "Extract Node Size" (15), "Feature Node Size" (10), "Maximum Spin System Size" (20), "Generate Network", and "View Network". A "Load Parameters" button is at the bottom of the "Parameters:" section, and a "Remove Selected Sample From List" button is at the bottom of the "Samples List:" section.

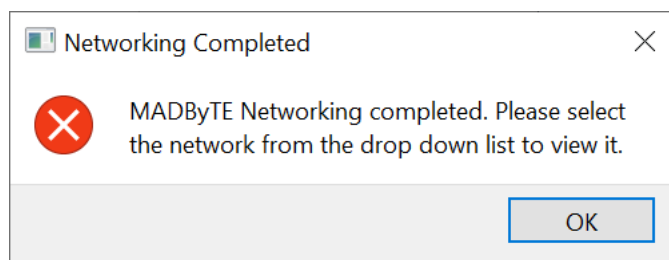
Users can customize the look of the finished network by altering the Extract Node Color and Size, the Spin Node Color and Size, and the Maximum Spin System Size values. Detailed information on these can be found in the user manual. For this example, we will use the default values.

To generate the network, click the Generate Network Button.

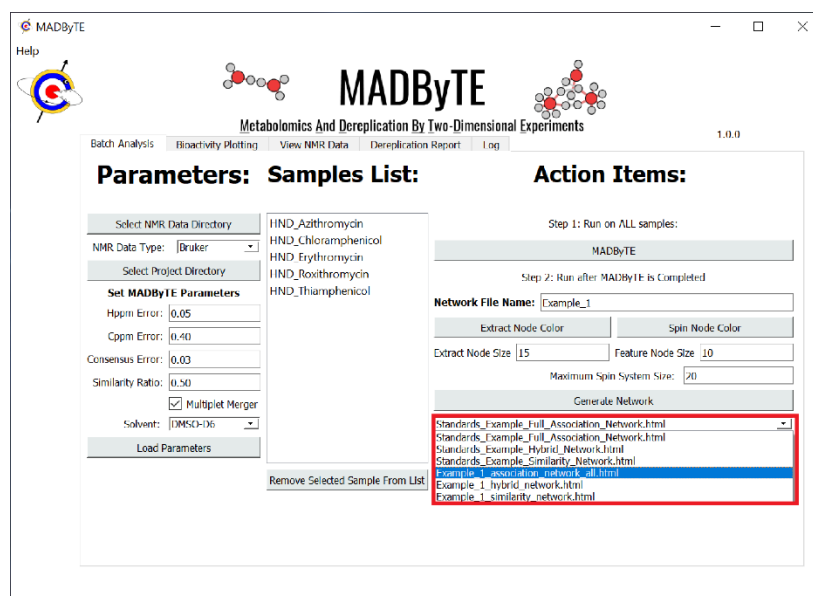


This screenshot is similar to the previous one, showing the MADByTE software interface. The "Network File Name" field is still highlighted with a red box. In this view, the "Generate Network" button in the "Action Items:" section is also highlighted with a red box. The "View Network" button is visible below it. The "Load Parameters" button is at the bottom of the "Parameters:" section, and the "Remove Selected Sample From List" button is at the bottom of the "Samples List:" section.

Upon completion, the following message will be displayed:



To view the resulting network, select it in the dropdown list and click View Network.



This opens the Network Viewer Plugin and displays the selected network. Users can interact with the network by hovering over nodes to see membership of each spin system as well as the identity of the spin system feature.

