

# MS Amanda for Proteome Discoverer 1.4

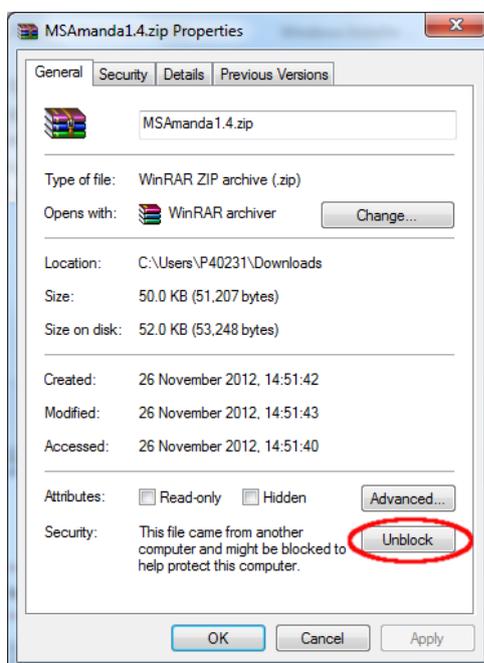
## User Manual

January 2013

### Installation of MS Amanda

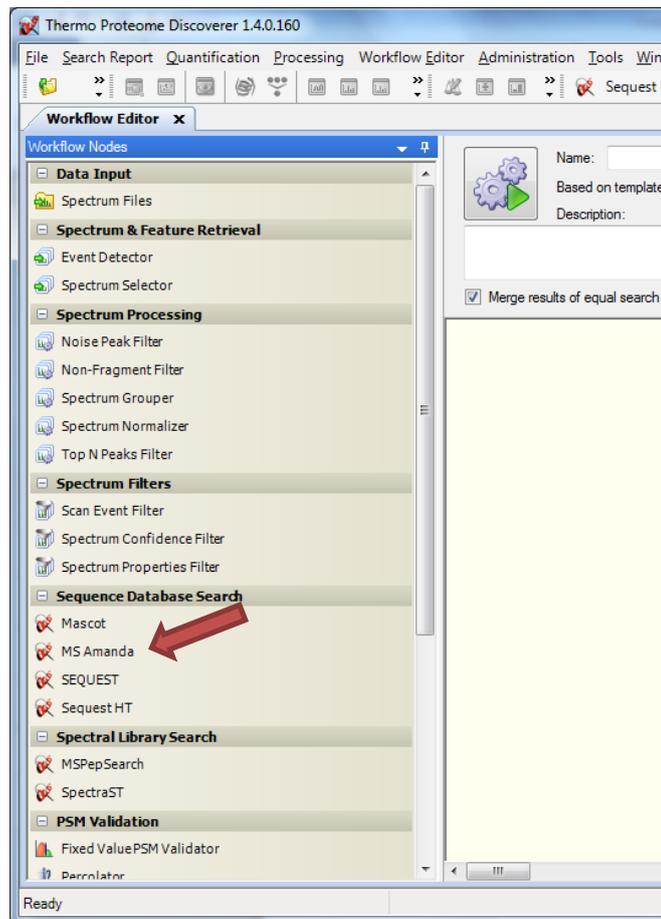
In order to install the MS Amanda node and achieve optimal performance, several steps have to be performed:

1. Download the .zip file from <http://ms.imp.ac.at/?goto=msamanda>
2. Right click on the downloaded .zip file and select the menu item "Properties" in the context menu
3. Click "Unblock" at the bottom right of the Properties window.



4. Click "OK" and close the Properties window.
5. Extract the .zip file
6. Shutdown Proteome Discoverer 1.4.
7. Copy all extracted files to the "Release" folder in the installation folder of Thermo Proteome Discoverer 1.4, which is for a standard installation something similar to "C:\Program Files\Thermo\Discoverer 1.4\System\Release".  
If you cannot find the installation path, right click on the Proteome Discoverer shortcut on your Desktop or in your start menu and select the menu item "Properties". The location of your installation is can be found in the "Target" description.
8. Run "AmandaInst.exe"

9. MS Amanda should now be successfully installed and appear as a node in the category “Sequence Database Search”:



## Parameters of MS Amanda

There are various parameters available to configure the MS Amanda scoring system:

### 1. Input Data

#### a. Protein Database

The sequence database MS Amanda should use for identifying peptides. A forward as well as a combined forward and reverse (decoy) database can be selected. Please select a database previously added through the Proteome Discoverer Fasta File interface.

*Please note: The first search on a new database or with a new digestion enzyme may take significantly longer than the second one as the MS Amanda scoring system digests and indexes every database file (for a selected enzyme and missed cleavage sites) only once at the first search. Further searches can then be performed considerably faster.*

#### b. Enzyme Name

The enzyme the database shall be digested with. Please select an appropriate enzyme.

*Please note: Selecting "No-Enzyme" will need – depending on the size of your protein database – a significant amount of available space on your hard disk (in contrast to other enzymes); moreover, searches will also take significantly longer.*

#### c. Missed Cleavages

The number of possible missed cleavage sites the database shall be digested with. Possible numbers are 0 - 4.

#### d. MS1 tolerance

The tolerated mass error for MS1 mass values (precursor masses). Possible units are "Da" and "ppm".

#### e. MS2 tolerance

The tolerated mass error for MS2 mass values (fragment masses). Possible units are "Da" and "ppm".

### 2. Static Modification

#### a. 1. Static Modification – 3. Static Modification

Various static (fixed) modifications can be specified through selection from the drop down box. They are applied to each instance of the specified residue.

#### b. 4. Static N-Terminal Modification & 5. Static C-Terminal Modification

In addition, static peptide N- or C-Terminal modifications can be specified through selection from the drop down box. They are applied to each instance of the specified peptide terminus.

### 3. Dynamic Modification

#### a. 1. Dynamic Modification – 5. Dynamic Modification

Various dynamic (variable) modifications can be specified through selection from the drop down box. They may or not be applied to an instance of the specified residue.

#### b. 6. Dynamic N-Terminal Modification & 7. Dynamic C-Terminal Modification

In addition, dynamic peptide N- or C-Terminal modifications can be specified through selection from the drop down box. They may or not be applied to an instance of the specified peptide terminus.

#### 4. Advanced Parameters: Additional Settings

a. **Max. No. of same mods**

The maximum number of possible equal modifications per peptide, even if more sites are available.

b. **Ion Settings**

This parameter specifies the fragment ions and neutral losses that are calculated. Possible ions are a, b, c, x, y, z, z+1, z+2, Int (internal fragments), Imm (immonium ions), -H<sub>2</sub>O (water loss), -NH<sub>3</sub> (ammonia loss). Additional combinations can be added as XML nodes in the file "Instruments.xml":



```
Instruments.xml x
<?xml version="1.0"?>
<!-- possible values are: "a", "b", "c", "x", "y", "z", "z+1", "z+2", "H2O", "NH3", "IMM", "z+1",
<instruments>
  <setting name="b,y">
    <series>b</series>
    <series>y</series>
  </setting>
  <setting name="b,y,-H2O,-NH3">
    <series>b</series>
    <series>y</series>
    <series>H2O</series>
    <series>NH3</series>
  </setting>
  <setting name="a,b,y,-H2O,-NH3,Imm">
    <series>a</series>
    <series>b</series>
    <series>y</series>
    <series>H2O</series>
    <series>NH3</series>
    <series>IMM</series>
  </setting>
  <setting name="a,b,y,-H2O,-NH3">
    <series>a</series>
    <series>b</series>
    <series>y</series>
```

c. **Max. Rank**

The maximum rank number that shall be reported to the result file.

d. **Peptide Cut Off Score**

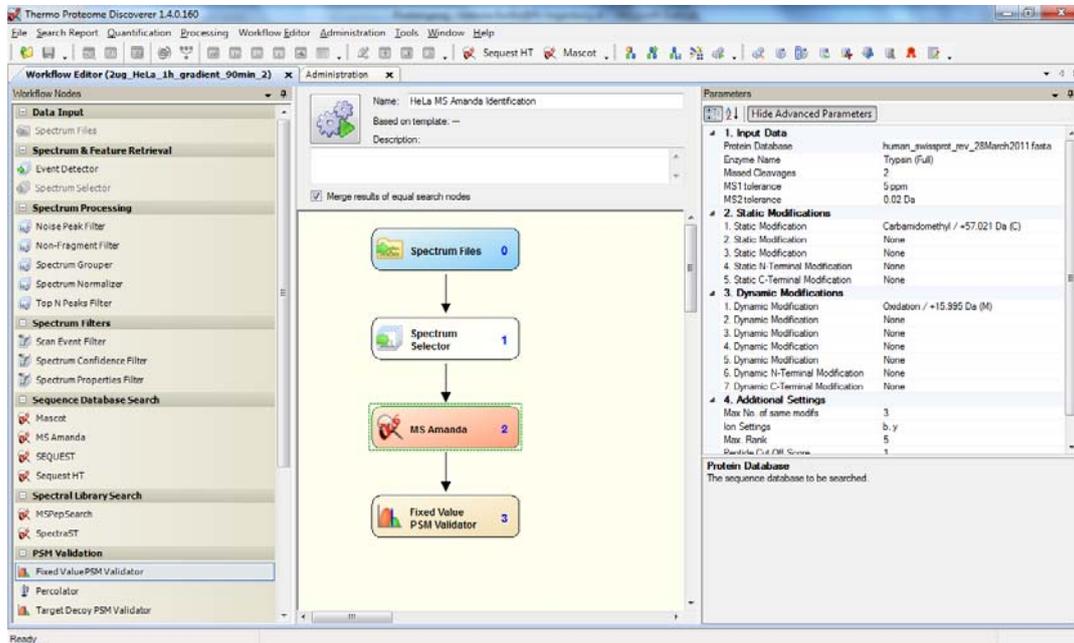
Only peptides with a score above this threshold are stored.

e. **Perform deisotoping**

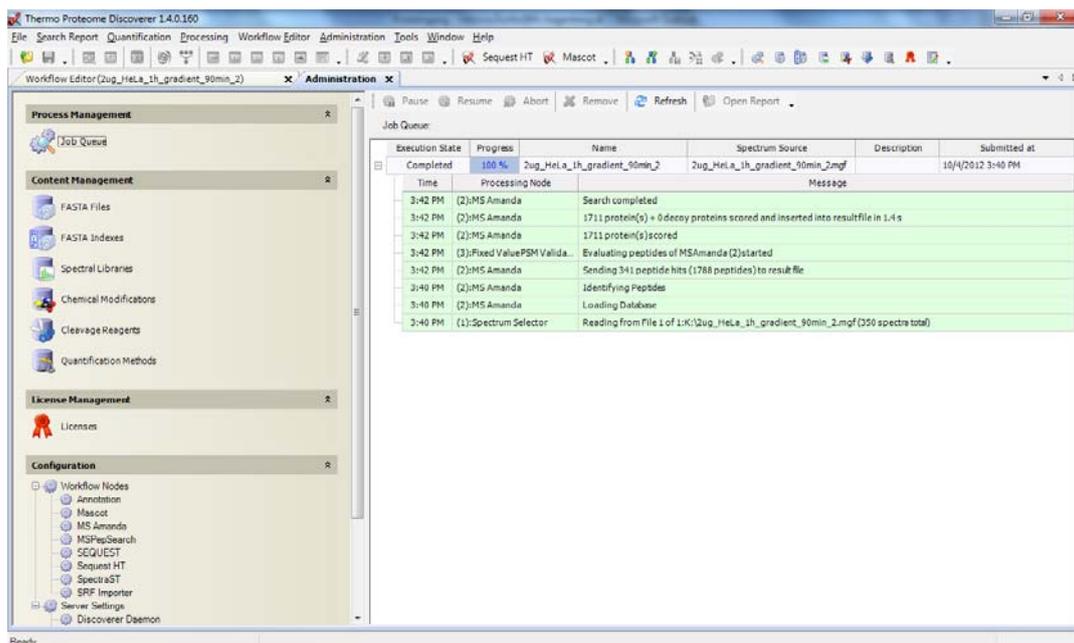
Defines whether a simple deisotoping shall be performed.

## Running MS Amanda

Running MS Amanda in Proteome Discoverer is similar to running a Mascot or SEQUEST search. Simply drag & drop the MS Amanda node into your workflow and connect it to its predecessor and successor nodes:

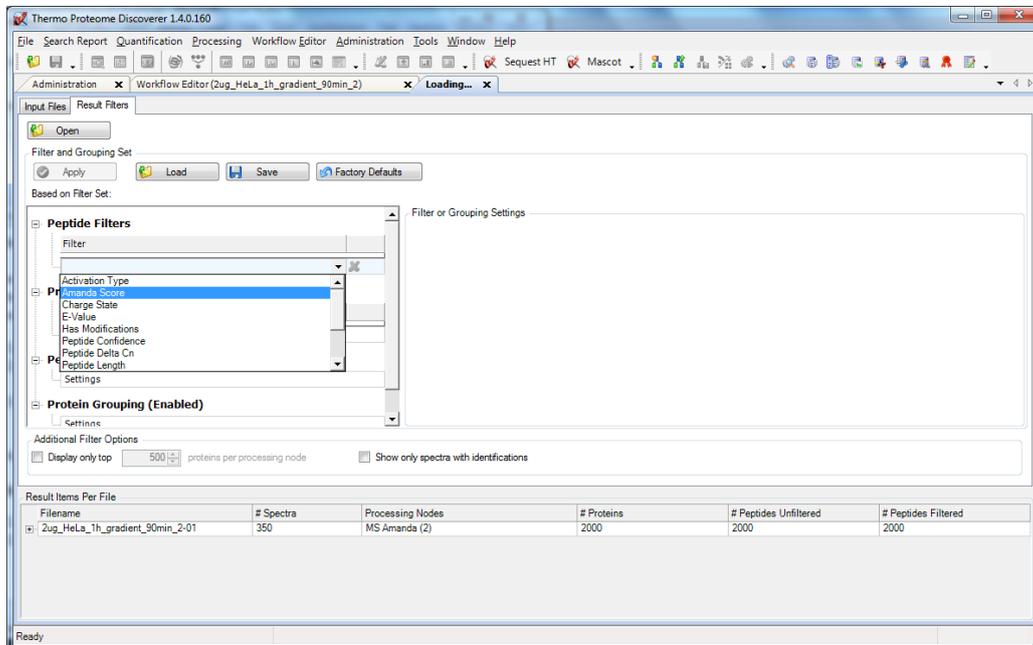


Afterwards, please set the parameters fitting your experiment composition and click the run button. The MS Amanda search will start immediately; after the run is finished you can open the report. *Please note: The first search on a new database or with a new digestion enzyme may take significantly longer than the second one, as the MS Amanda scoring system digests and indexes every database file (for a selected enzyme and missed cleavage sites) only once at the first search. Further searches can then be performed considerably faster.*

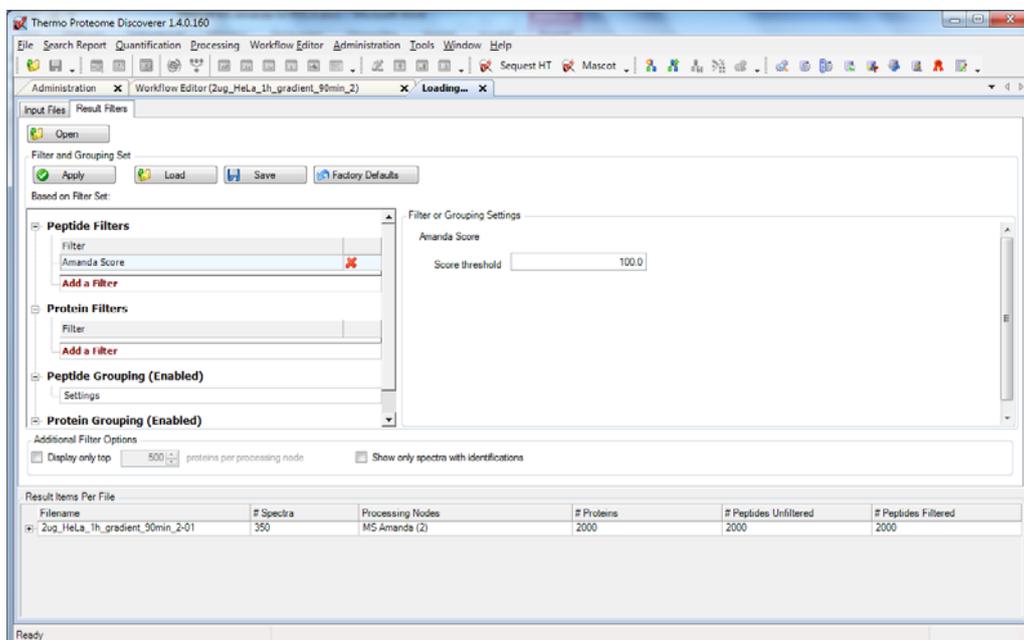


## Filtering results obtained with MS Amanda

When opening the report you can filter the peptide spectrum matches obtained with MS Amanda on two different scores provided by MS Amanda, namely the *Amanda Score* and the *E-Value*. In the “Result Filters” tab in the “Peptide Filters” selection box you find the entries “Amanda Score” and “E-Value”:



After selecting one of these entries you can filter your search results for the selected score:



Please note: Filtering using an **Amanda Score** of 100 as threshold means that all PSMs having a score **above or equal to 100** are selected, all those beyond are filtered out; filtering using an **E-Value** of 0.01 as threshold means that all PSMs having an **e-value below or equal to 0.01** are selected, all those above this threshold are filtered out.