Use the Metabolika module to visualize metabolic pathways and to create, store, export, and process Metabolika drawing files.

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- Overview
- Draw a metabolic pathway
- Save the drawing as a Metabolika file

# **Overview**

A Metabolika drawing file consists of chemical structures interconnected by arrows and represents a metabolic reaction or a series of reactions. You can send the pathway structures individually or in a batch to other Mass Frontier modules where they are used for data curation, substructure searches, fragment generation, and so on.

In the Metabolika module, you can add and edit chemical structures, connect them with various arrow types, and annotate them with custom text and labels. You can import structures from supported file types or create them by using the internal structure editor. You can export the pathway structures to structure grids and use them in other Mass Frontier modules. For example, you can use the structures in a structure grid to generate a set of XIC chromatograms in the Chromatogram Processor module.

This tutorial shows you how to draw the metabolic pathway for the bacterial decomposition of the antibiotic sulfamethoxazole by Pseudomonas psychrophila HA-4<sup>1</sup> and save the drawing as a Metabolika drawing file.



<sup>1</sup> Jiang, B., Li, A., Cui, D., Cai, R., Ma, F., & Wang, Y. (2014). Biodegradation and metabolic pathway of sulfamethoxazole by Pseudomonas psychrophila HA-4, a newly isolated cold-adapted sulfamethoxazole-degrading bacterium.

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# **Draw** a metabolic pathway

To draw the metabolic pathway for the bacterial decomposition of sulfamethoxazole, follow these topics in order:

- 1. Open a new pathway document
- 2. Add structures to the pathway
- 3. Label structures
- 4. Add Directional arrows to the pathway
- 5. Add arrow captions
- 6. Add elemental formulas

### **Open a new** pathway document

- To open a new pathway document
- To open a new pathway document
   Open the Mass Frontier application by double-clicking its desktop icon, or by choosing Thermo Mass **Frontier 8.1 > Mass Frontier 8.1** from the Windows<sup>™</sup> Start menu.

The application opens to the Mass Frontier startup window or the Modules & Tools toolbar.

Metabolika button in the

- 2. In the Modules & Tools toolbar, click Metabolika (Figure 1).
  - Figure 1. Mass Frontier startup window

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Note If you clear the Show this Window Next Time check box (below the About option), the next time you open the application, it opens to the Modules & Tools toolbar.

A new document named New Pathway 1.metabolikadrw opens as a tabbed page with a toolbar on the left, a drawing area in the middle, and a set of tabbed pages on the right that are opened to the Properties page. Right-clicking the drawing area opens a shortcut menu (Figure 2).

Opens the Structure Editor dialog box. Tabbed pages New Pathway page Metabolika toolbar M F 1 A 🗋 = 📂 r 🗰 Metabolika Search 7 \_ D X 🤐 online 🔹 📳 MB Start Marke Settin Modules & Tools Search QQQ 82 ٢  $\square$ X ٦. Ne Import Paste Structure Structures Delete Select Pan **~** Show Show Structures File Copy & Paste Send Edit Zoom & Pan Viev Metabolika - New Pathway 1.metabolikadrw X . Dr ~ Properties Metadata Errors Marks Pathways Explorer Add Structure k ₩ Reset Individual Reset Default 2 Add Formula B= ■ Search Add Standard Text T S CHN Draw Straight Arrow Merge Text and Arrow Is Reversible Alianment ~ -11 Edit Structure Edit Standard Text 医白 金 Edit Arrow Caption Mode ~ Reshape ଘ ର Is Locked Show Structure as 1 🗐 🖽 🔗 Zoom and Pan ۲ Сору Paste . Delete Select All Drawing area tools Drawing area Shortcut menu

Figure 2. New pathway page opened in the Metabolika module

Leave the New Pathway page open and go to the next topic "Add structures to the pathway."

Add structures to the pathway

There are several ways to add structures to the pathway. You can load a structure from a 2D structure file, draw a structure by using the Structure Editor, or send structures from a Structure Grid or another .metabolikadrw file.

To add the structures and some of the other pathway objects to the drawing area, follow these procedures in order:

- 1. Add a structure from a MOL file
- 2. Send structures from a structure grid
- 3. Copy a Metabolika pathway

**Note** To show you how to add arrows and text to a metabolic pathway, this topic instructs you to copy only the structures from an existing Metabolika drawing file instead of all the pathway elements.

#### Add a structure from a MOL file

To add the parent compound—sulfamethoxazole—to the pathway, use the MOL file provided in the Demo Data folder.

#### To add a structure from a MOL file to the pathway

- 1. In the New Pathway document, do either of the following:
  - In the Draw toolbar at the left, click the Add/Edit Structure icon, (Figure 2).

-or-

• Right-click the drawing area and choose Add Structure (Figure 2).

The Structure Editor opens as a dialog box with a general toolbar at the top and three structure-specific toolbars at the left (Figure 3).

Figure 3. Structure Editor dialog box

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- 2. In the toolbar at the top of the Structure Editor, click the **Open** icon, *P*.
- 3. Browse to the following folder:

drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.0\Demo Data\Structures

4. Select the following 2D structure file-sulfamethoxazole.mol.

The structure for sulfamethoxazole appears in the Structure Editor's drawing area.

5. Click OK.

The Structure Editor closes and the structure appears as a structure object in the drawing area of the New Pathway page.

6. Use the left mouse button to move the structure in the drawing area.

Figure 4. Sulfamethoxazole added to the drawing area of a New Pathway page



Leave the New Pathway page open and go to the next topic, "Send structures from a structure grid."

#### Send structures from a structure grid

The SMX Decomposition.metabolikadrw file in the Demo Data folder contains the metabolic pathway for sulfamethoxazole. In this topic, you send the structures in the demonstration drawing to a Structure Grid page, and then send the structures to the New Pathway page where you are creating the new drawing.

**Note** You can copy all or part of a Metabolika drawing to another Metabolika drawing or send one or more structures in a Metabolika drawing to a Structure Grid page.

#### \* To send structures from a Metabolika drawing file to a structure grid

- 1. Open the example Metabolika drawing that contains the pathway of interest as follows:
  - a. In the Metabolika toolbar, click New.

A second New Pathway page opens to the right of the current New Pathway page.

- b. In the Metabolika toolbar, click Import.
- c. Browse to the following folder, select SMX Decomposition.metabolikadrw, and click Open.

drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.0\Demo Data\Metabolika

2. To send the structures on the second New Pathway page to a structure grid, in the Send Group of the Metabolika toolbar, click **Structures**, and then select **New Structure Grid** from the dropdown list.



A new Structure Grid page with 25 cards opens to the right of the second New Pathway page. **Figure 5.** New structure grid with 25 cards



 In the Send To group of the Structure Grid toolbar, click Structures and select Metabolika-New Pathway 1.metabolika.drw. (This is the Metabolika window that currently only includes the parent compound.)

Figure 6. Sending the structures to the New Pathway 1 drawing page



4. At the prompt, click Yes multiple times until the prompt closes and the structures appear.

Note You have to click Yes multiple times because the structure grid contains replicate cards.

The structures from the Structure Grid appear on the Metabolika - New Pathway 1.metabolikadrw page, but they are very small.

- 5. To enlarge the structures, click the **Zoom Reset** icon, <sup>(h)</sup>, in the Zoom & Pan group of the Metabolika toolbar.
- 6. For this tutorial, delete the new structures, and go to the next procedure, "Copy a Metabolika pathway."

You now have no structures on the New Pathway 1 page where you are creating the new drawing. Leave the New Pathway 1 page open and go to the next topic, "Copy a Metabolika pathway."

#### Copy a Metabolika pathway

#### To copy a pathway from another New Pathway page

1. To open the New Pathway 2 page that contains the full metabolic pathway for sulfamethoxazole, click the **New Pathway 2** tab.

2. At the left of the drawing area, under Mode, click the **Rectangular Selection** tool, . , or the **Lasso Selection** tool, , and then drag the pointer across or around all the structures in the pathway, with the exception of the parent structure, as shown in Figure 7.



Figure 7. Portion of pathway selected by dragging the Rectangle Selection tool across the structures

- 3. In the Metabolika toolbar, click Copy, and then choose Copy Selection from the dropdown menu.
- 4. To return to the New Pathway 1 page where you are creating the new drawing, click its tab.
- 5. In the Metabolika toolbar, click **Paste**, and then choose **Paste Scheme** to paste the selection to the New Pathway 1 page.



Figure 8. Pasting the selection to the New Pathway 1 page

- 6. Before you go to the next procedure, do the following:
  - a. Deselect the structures on the New Pathway 1 page.
  - b. Add the parent compound above the partial pathway by following the instructions in "Add a structure from a MOL file."
  - c. Move the parent compound, sulfamethoxazole, to the top of the drawing area.
- 7. Close the Structure Grid page.

Structure Grid 1

8. Close the New Pathway 2 page.

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	Close

Leave the New Pathway 1 page open and go to the next topic, "Label structures."

Label structures

You can annotate a structure by adding standard text or a label—however, if you want the text to be part of the structure object and move together with the structure, annotate the structure by adding a label.

#### ✤ To add a label to a structure

- 1. In the drawing area of the New Pathway page, select the parent structure at the top.
- 2. On the Properties page to the right, click the browse icon, ..., to the right of the Text box in the Label area (Figure 9).

Figure 9. Parent compound selected

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Opens a text entry boxfor creating a label

A text entry box opens.



3. Type the label and click **OK**. For parent structure in this tutorial, type **sulfamethoxazole**. The label appears below the structure.



**Note** To change the properties of a label after adding it to a structure object, select the structure object and modify the label's properties on the Properties page.

Leave the New Pathway 1 page open and go to the next topic, "Add Directional arrows to the pathway."

### Add Directional arrows to the pathway

Arrows indicate the direction between reactants and products. Follow the first procedure to add the three missing directional arrows to the pathway. The remaining procedures are optional.

#### To add the missing directional arrows

- 1. To draw a straight arrow from the parent compound to the hydrolysis product on the right, under Draw, click the Add/Connect to Straight/Arc Connector icon, .
- 2. Point to the parent structure, and then drag the mouse toward the first hydrolysis product on the right. By default, the arrow is locked to the parent compound, but not the hydrolysis product.
- 3. Right-click the arrow and choose Is Locked.



Leave the New Pathway 1 page open and go to the next topic, "Add arrow captions" on page 11.

(Optional) To modify the arrows in a Metabolika pathway, follow these procedures:

- To move an arrow
- To change an arrow's angle
- To change an arrow's curvature
- To straighten a curved arrow
- To make an arrow bidirectional to represent a reversible reaction
- To merge arrows

#### To move an arrow

1. Select the arrow and hold down the left mouse button to display the move pointer (+,+).



2. Drag the arrow to another location.

#### To change an arrow's angle

- 1. Select the arrow to display its anchor points, and then point to an end anchor point.
- 2. When this pointer (1) appears, drag the end of the arrow up or down as needed.

#### To change an arrow's curvature

- 1. Select the arrow to display its anchor points, and then point to one of its two internal anchor points.
- 2. When this pointer (1) appears, drag the pointer arrow up or down as needed.



#### To straighten a curved arrow

Right-click it and choose **Reshape**.

**\*** To make an arrow bidirectional to represent a reversible reaction

Right-click it and choose Is Reversible.

#### To merge arrows

- 1. Reshape the arrows into curves.
- 2. Overlay the curved arrows, and then select one of the curves.
- 3. Point to any of the blue squares.
- 4. When this pointer ('\$\vec{L}') appears, drag the selected curve until the red square anchor points appear on the second curve. Then, release the mouse button.



**Note** The shape of your merged curves might differ from those shown above.

5. To straighten one of the merged arrows, right-click it and choose Reshape.

### Add arrow captions

Add arrow captions to the two hydrolysis steps at the top of the pathway.

#### To add an arrow caption

1. Right-click the arrow for the first hydrolysis reaction at top left of the pathway, and then choose **Edit Arrow Caption**.

A dialog box opens.

2. Type text and click **OK**.

For this tutorial, type **hydrolysis**.

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3. Right-click the arrow for the second hydrolysis reaction, choose **Edit Arrow Caption**, type **hydrolysis**, and click **OK**.



Leave the New Pathway 1 page open and go to the next topic, "Add elemental formulas" on page 12.

### Add elemental formulas

The metabolic pathway that you copied from the SMX Decomposition.metabolikadrw file contains structures for small molecules, such as water and carbon dioxide. For simplicity, you can replace small structures with elemental formulas.

#### To add elemental formulas to a drawing

- 1. Right-click in the drawing area and choose Add Formula.
- 2. Type the Formula in the Formula dialog box.

Formula		×
H2O		
	OK	Cancel

#### 3. Click **OK**.

The formula appears in the drawing area with the numeric value for each element inserted as a subscript, for example,  $H_2O$ .

Leave the New Pathway 1 page open and go to the next topic, "Save the drawing as a Metabolika file."

## To save the new metabolic pathway drawing

- 1. In the File group of the Metabolika toolbar, click Save, and then select Save As.
- 2. Browse to an appropriate folder.

If you have not set already set up folders for your Metabolika pathway files, the Save Metabolika File dialog box opens to the Demo Data\Metabolika folder.

- 3. In the File Name box, type A New Pathway.
- 4. Click Save.

#### To view a Metabolika pathway from the Pathways Explorer

- 1. In the File group of the Metabolika toolbar, click New.
- 2. Close the A New Pathway file that you created and saved in this tutorial.
- 3. To the right of a Metabolika New Pathway document, click the **Pathways Explorer** tab (see Figure 2 on page 3). The Pathways Explorer page appears.
- 4. To view any of the pathways that are provided with the application, click the **Choose Default Folder** icon, **(**Figure 10).

# Save the drawing as a Metabolika file

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5. In the pathway list, select a pathway.

The selected pathway opens as a tabbed page.

- 6. On the Pathway Explorer page, click the **Choose Custom Folder** icon, 🧖.
- 7. Browse for the folder where you saved the A New Pathway.metabolikadrw file and click OK.
- 8. In the files list, select the file that you want to view or edit.

The selected pathway opens as a tabbed page.

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