PATHWAY STUDIO

ELSEVIER

Guide to Building Pathways in ChemEffect® and DiseaseFx™ using Pathway Studio® Web

Pathway Studio Web Version 2.0

This guide is for ChemEffect and DieaseFx database users who are already familiar with and received training on Pathway Studio Web and the Mammalian database. If you have not received any training, please refer to the **Guide to Building Pathways in Mammal using Pathway Studio Web** prior to reviewing this document. You can find support material at the Elsevier support site: http://www.elsevier.com/online-tools/pathway-studio/customer-support#guides-and-manuals

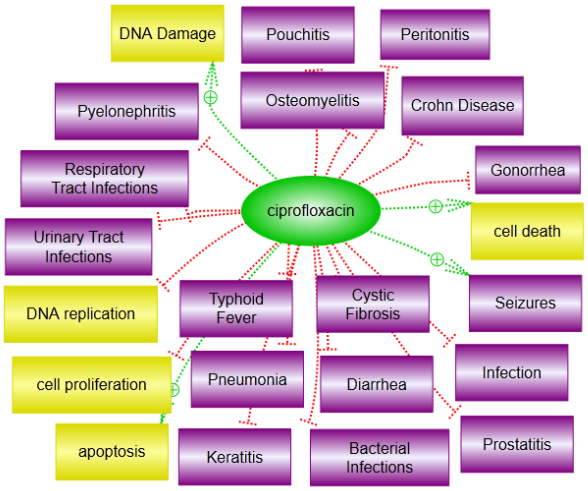
New information is provided with the ChemEffect Database

In the Mammalian database, the small molecule collection includes naturally occurring small molecules and their relations to proteins, functional classes, and complexes. The ChemEffect database includes both naturally occurring and non-naturally occurring small molecules (i.e. drugs). Accordingly, the number of relations between small molecules and proteins, complexes, and functional classes is higher with the addition of the ChemEffect database.

The ChemEffect database includes relations between small molecules and diseases and small molecules and cell processes. These relations include:

Entity	Relation	Entity
Small Molecule	Regulation	Cell Processes
Small Molecule	Regulation	Disease

Below is an example of relations associated with small molecules in the ChemEffect database. In this example Diseases and Cell Processes are regulated by the small molecule ciprofloxacin.



The ChemEffect database contains a collection of 22 curated toxicity pathway representing highly referenced toxicological processes found in the current scientific literature. These toxicity pathways compliment the signaling and metabolic pathways included in the ResNet mammalian database. Toxicity pathways can be found in the Elsevier Pathways folder and constitute an additional gene set category available for use in the enrichment algorithms, specifically Find Pathways/Groups Enriched with Selected Entities (Fisher's Exact Test) algorithm and the Gene Set Enrichment Analysis algorithm.

Toxicity terms are included in the Disease entity collection annotation. You can examine the disease terms and the disease term aliases for familiar toxicity concepts in the properties view.

Examples of Pathway Building Options Utilizing the ChemEffect Database

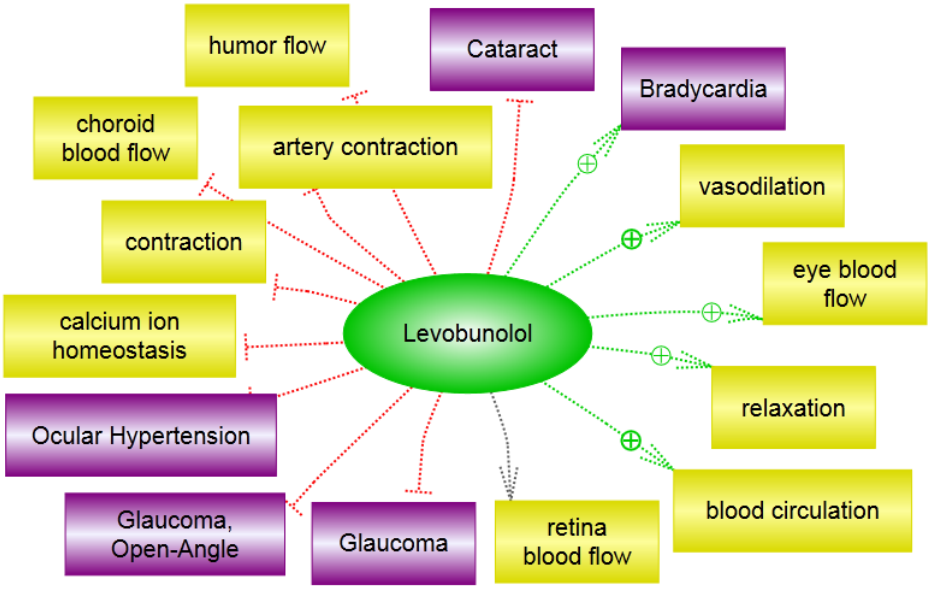
1. Identify Known and Potential Drug Effects

Mine the database for known regulatory associations between a small molecule and diseases and/or cell processes.

Steps to follow:

- Add a small molecule to a new pathway view and select the small molecule.
- Wizard settings: **algorithm**: Expand Pathway; **directionality**: downstream; **entity type**: disease and cell process; **relation type**: regulation.
- Optional: Style>Active Style Sheet>By Effect
- (Optional: remove all relations with a reference count of less than 5 to increase confidence)

Examine the diseases and cell processes in the resulting network.



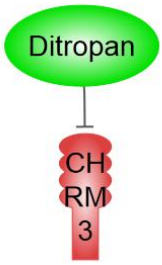
2. Predict Potential Drug Effects

Predict potential drug effects by identifying drug targets and examining the Cell Processes and Diseases associated with those drug targets.

Steps to follow:

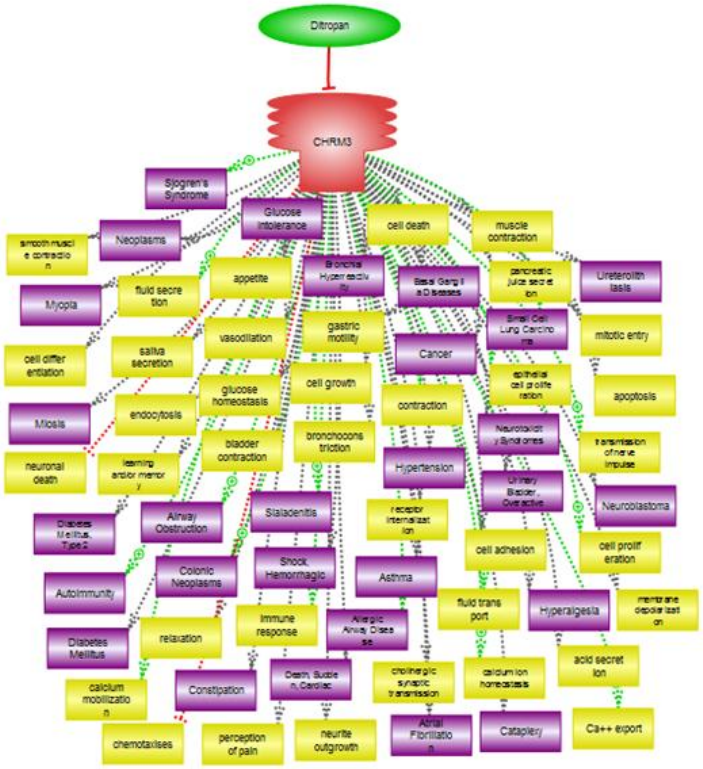
1. Examine proteins that are negatively impacted by the small molecule

- Add a small molecule to a new pathway view and select the small molecule.
- Wizard settings: **algorithm**: Expand Pathway; **directionality**: downstream; **entity type**: protein; **relation type**: directregulation. Find proteins that are negatively regulated.



2. Now examine the diseases and cell processes associated with this target protein.

- Select the protein.
- Wizard settings: **algorithm**: Expand Pathway; **directionality**: downstream; **entity type**: disease and cell process; **relation type**: regulation.
- Optional: Style>Active Style Sheet>By Effect
- (Optional: remove all relations with a low reference count to increase confidence)



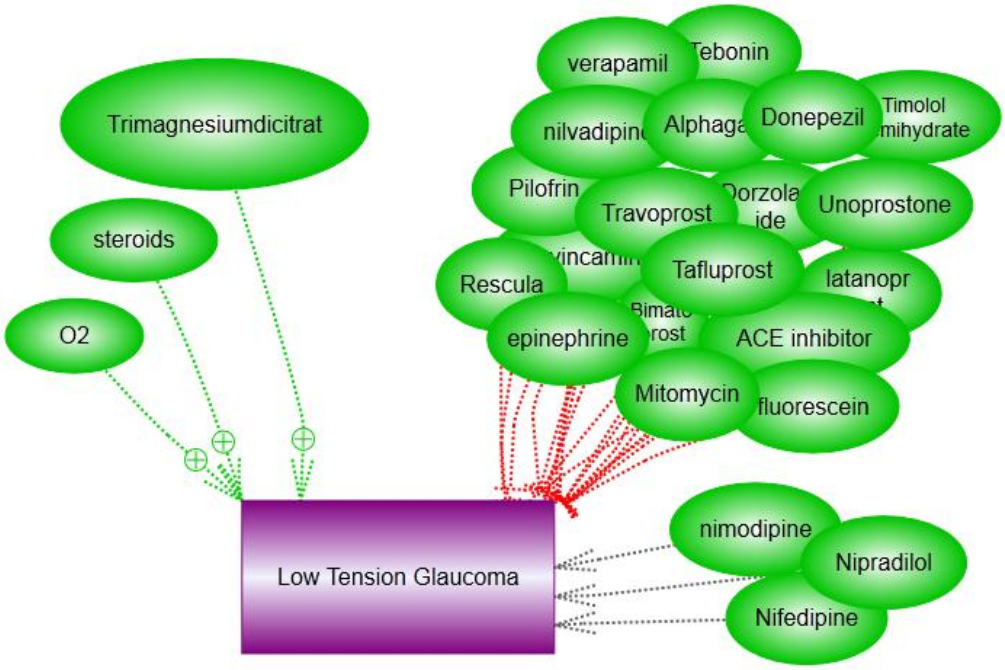
3. Find Compounds Associated With a Specific Toxicity

Find small molecules in the database that have a positive regulation relation with toxicity.

Steps to follow:

- Add toxicity term to a new pathway view and select the entity.
- Wizard settings: **algorithm**: Expand Pathway; **directionality**: upstream; **entity type**: small molecule; **relation type**: regulation.
- Style>Active Style Sheet> By Effect

Examine the small molecules that have a positive effect on the disease/toxicity term.
(Optional) Remove relations with a low reference count to increase confidence.

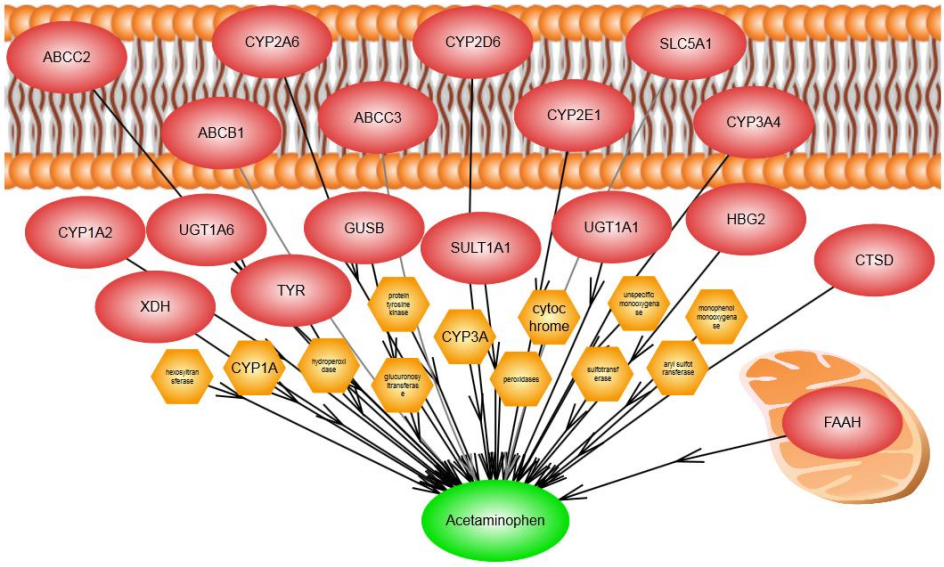


4. Find Enzymes and Protein Transporters Involved in the Metabolism of a Drug

Find proteins, complexes, and functional classes upstream of a drug with relation types of ChemicalReaction or MolTransport.

Steps to follow:

- Add small molecule to a new pathway view and select the small molecule
- Wizard settings: **algorithm**: Expand Pathway; **directionality**: upstream; **entity type**: protein/complex/functional class; **relation type**: chemicalreaction and moltransport.

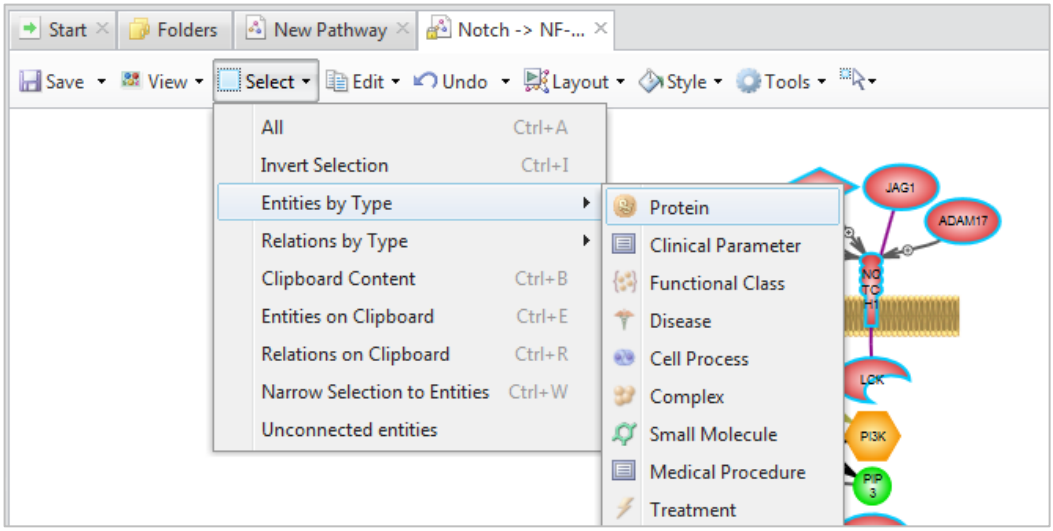


5. Find Drugs Targeting Multiple Proteins in a Select Pathway

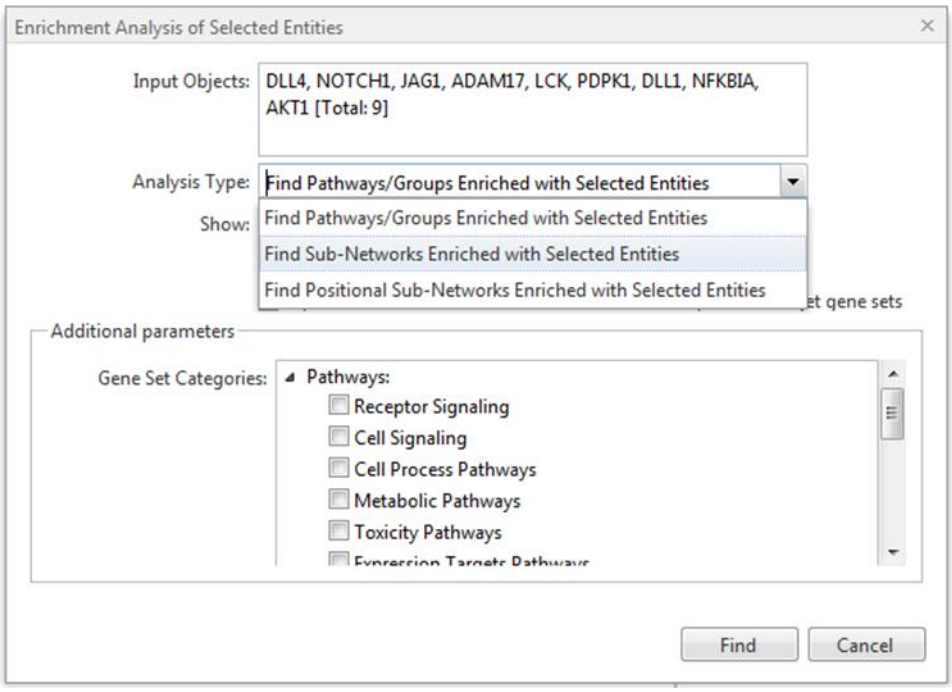
In order to take advantage of the knowledge environment enriched with small molecules and their effects on protein targets, Elsevier added an additional preset option to run enrichment algorithms on sub-networks. This option enables you to identify small molecules that regulate the expression of proteins from a list or an experimental dataset by using the preset option “Chemical Expression Targets.” This new preset available with ChemEffect is used in the following workflow.

Steps to follow:

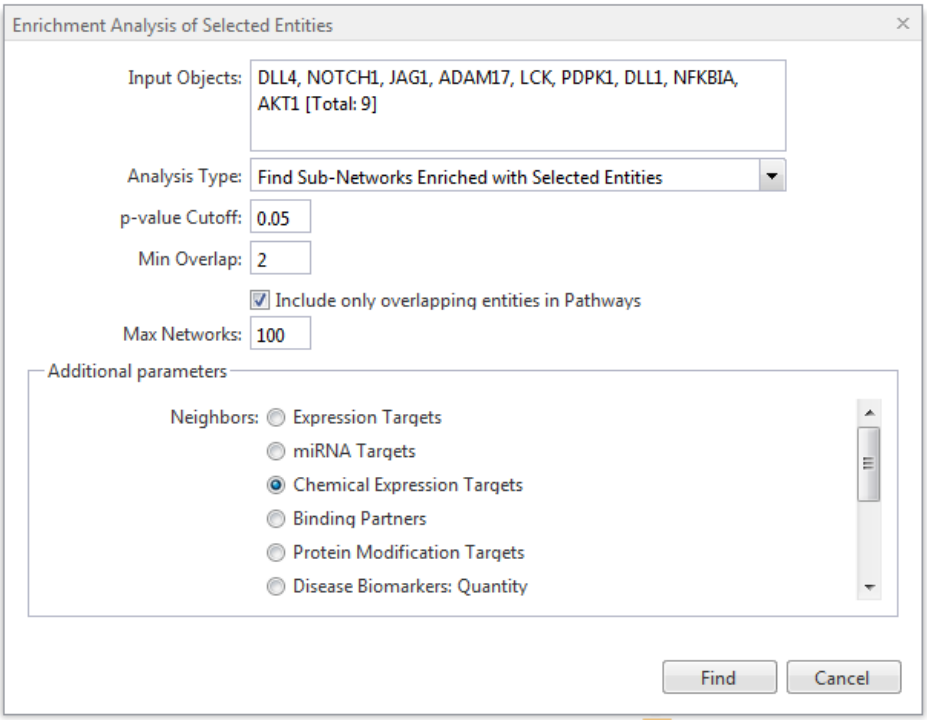
- Open a pathway of interest. In this example we will use Notch->NFkB Signaling pathway
- Select the protein entities in the pathway by using Select > Entities by Type > Protein



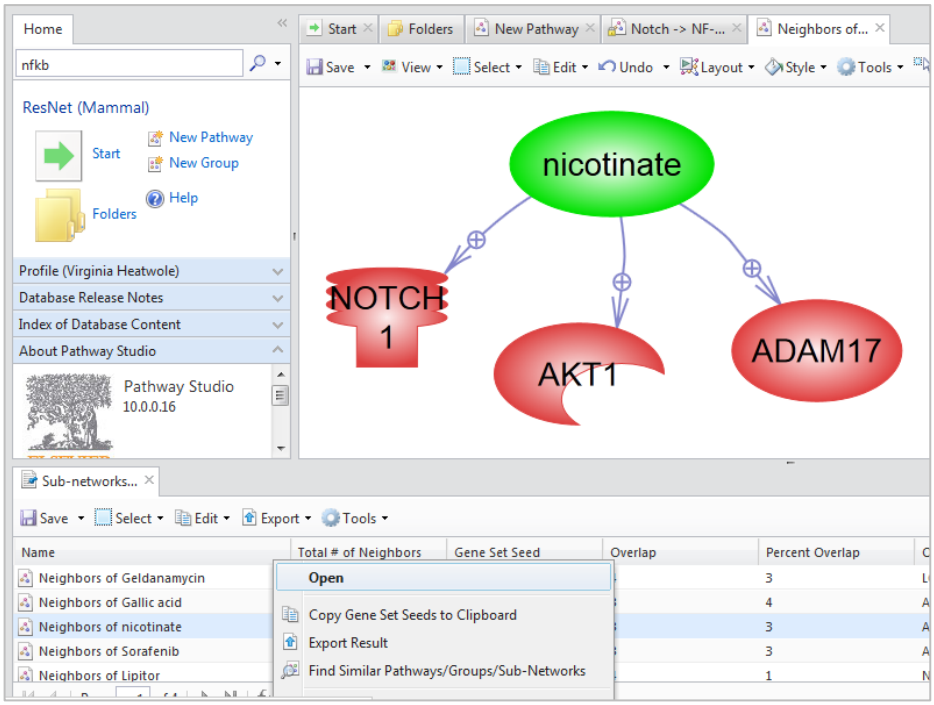
- From the Tools menu select Enrichment Analysis of Selected Entities
- In the Enrichment Analysis dialog, select Find Sub-Networks Enriched with Selected Entities from the drop down menu, and in additional parameters, select Chemical Expression Targets. Alternatively: In the custom settings you can also select DirectRegulation.



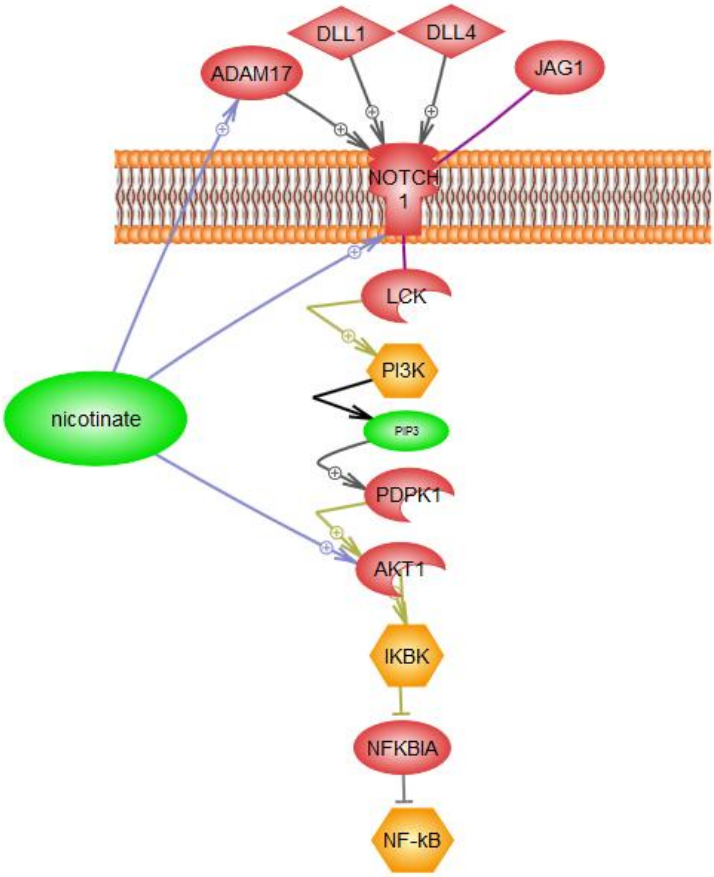
- Check the box “Include only overlapping entities on Pathways”



- Examine your results table and open a sub-network of interest.



- Select the entire sub-network and paste it in the graph view containing your pathway.



New information is provided in the DiseaseFx database

In the ResNet Mammalian database, disease relationships were limited to proteins. The DiseaseFx database expands the content of disease centric information by providing more types of relationships between disease and proteins as well as the introduction of new relation types between diseases and small molecules/functional classes/clinical trials. DiseaseFx relationships with diseases are tuned to be very specific to the context of the reference and are described by the following relationship types.

Entity-Entity Relation Type	New Relation Name	Sub-Relation Types*
Disease – Protein/Complex/Functional Class		
Changes in abundance/activity/expression of a gene/protein in a disease state	Quantitative Change	Field Name: Quantitative Type Sub-categories: Expression Abundance Activity
Genetic changes in a gene in a disease state such as gene deletions, amplifications, mutations or epigenetic changes	Genetic Change	Field Name: Change Type Sub-categories: Gene Deletion Mutation Gene Amplification Epigenic Methylation
Identification of proteins/complexes/functional classes/metabolites that are prognostic or diagnostic biomarkers for a disease	Biomarkers	Field Name: Biomarker Types Sub-categories: Diagnostic Prognostic
Changes in a protein's post-translational modification status or alternative splicing events associated with a disease	State Change	Field Name: Change Type Sub-categories: Alternative Splicing Phosphorylation
Disease – Small Molecule		
Changes in abundance of a small molecule in a disease state	Quantitative Change	Field Name: Quantitative Type Sub-categories: Expression Abundance Activity

Identification of small molecules that are prognostic or diagnostic biomarkers for a disease ***(limited to naturally occurring metabolites)***	Biomarkers	Field Name: Biomarker Types Sub-categories: Diagnostic Prognostic
Disease – Cell Process		
Different types of functional associations between a disease and a cellular process or another disease	Functional Association	(no sub-types)
Disease/Cell Process – Small Molecule		
Disease/cell process relationship representing clinical trials conducted for a drug against a disease (from ClinicalTrials.gov)	Clinical Trials	(no sub-types)

Table 1. Summary of additional relationships found in the DiseaseFx database.

** Sub-relation types are indicated in the annotation for each relation, in the field indicated above. Sub-relation types can be used to filter while building a network or upon examination of a completed network.*

Included in the DiseaseFx database is the Ariadne Expression Targets Pathway Collection. While the Elsevier Signaling Pathways start with signals and drill down to transcription factors, Elsevier's 386 Expression Targets Pathways go one step further and show the major expression targets of these transcription factors. This pathway collection will complement the Receptor Signaling, Cell Process, Cell Process Regulation and Metabolic pathways provided in the ResNet database and augment enrichment analysis in Pathway Studio by providing yet another collection of highly curated pathway data. The option to use these pathways in analysis is provided in the Gene Set Categories for the Fisher's Exact Test as well as the Gene Set Enrichment Algorithm.

Examples of Pathway Building Options Utilizing the DiseaseFx Database

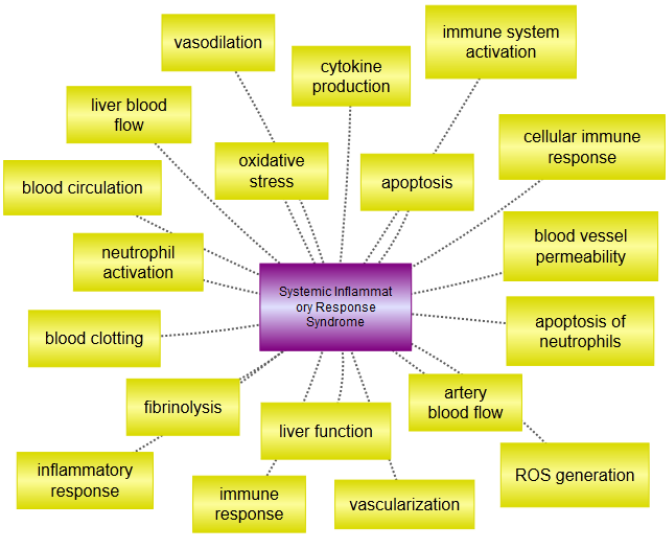
While the addition of the DiseaseFx data to the ResNet Mammalian database does not fundamentally change the functionality of Pathway Studio, it does allow for the user to ask additional biological questions that utilize the new data and relation categories. A few simple DiseaseFx specific workflows are demonstrated here:

1. Identify Cellular Processes Impacted in a Specific Disease State

Mine the database for cellular processes with functional association relations to a disease of interest

Steps to follow:

1. Identify the disease of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select "Build Pathway" and follow the wizard:
   - Step 1. Select algorithm "Expand Pathway"; Objects Directions: all; then select "Next,"
   - Step 2. From the entity list select "CellProcess," and from the relations list select "FunctionalAssociation." Select "Next."
   - Step 3 Select "Finish."

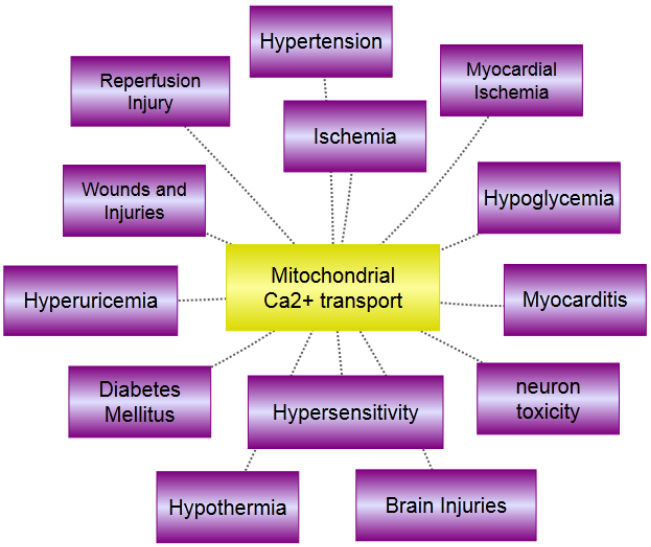


2. Identify Diseases Known to be Associated with a Particular Cellular Process

Mine the database for diseases with a functional association relation to a specific cell process

Steps to follow:

1. Identify the cell process of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select "Build Pathway" and follow the wizard:
   - Step 1. Select algorithm "Expand Pathway"; Objects Directions: all; then select "Next,"
   - Step 2. From the entity list select "Disease," and from the relations list select "FunctionalAssociation." Select "Next."
   - Step 3 Select "Finish."

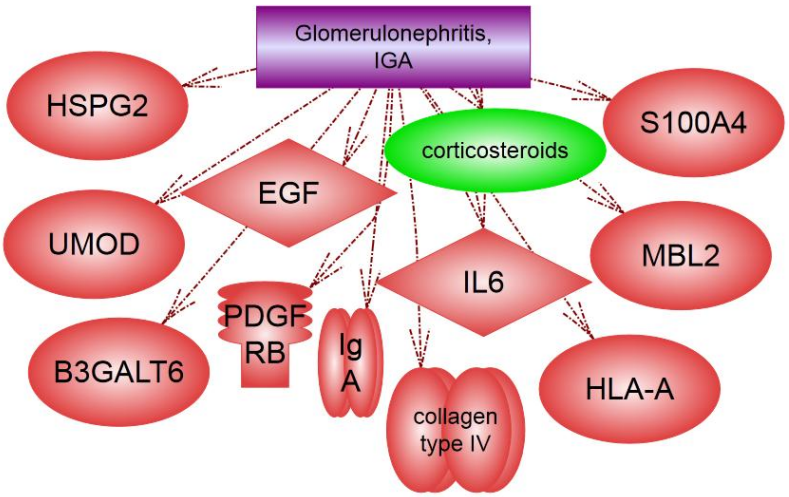


3. Identify Biomarkers for a Specific Disease

Mine the database for proteins/complexes/functional classes/ small molecules with a functional biomarker relation to a specific disease

Steps to follow:

1. Identify the disease of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select “Build Pathway” and follow the wizard:
   - Step 1. Select algorithm “Expand Pathway”; Objects Directions: downstream (arrow to the right); then select “Next,”
   - Step 2. From the entity list select “protein/complex/functionalclass/smallmolecule,” and from the relations list select “Biomarker.” Select “Next.”
   - Step 3 Select “Finish.”

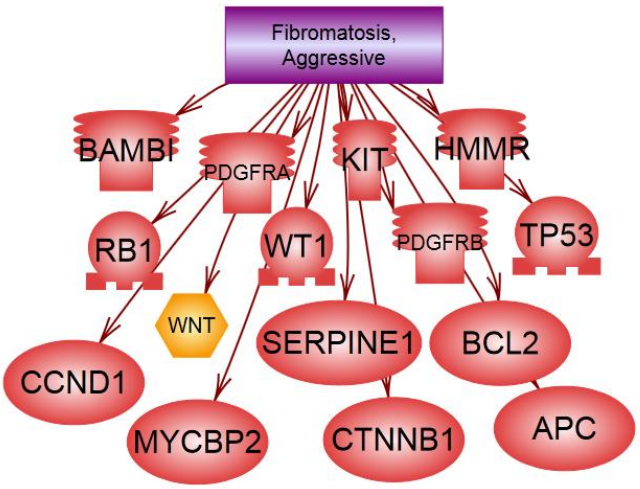


4. Identify Genetic Mutations in a Gene Associated with a Disease

Mine the database for genes with deletions, amplifications, mutations or epigenetic changes associated with a specific disease

Steps to follow:

1. Identify the disease of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select "Build Pathway" and follow the wizard:
   - Step 1. Select algorithm "Expand Pathway"; Objects Directions: downstream (arrow to the right); then select "Next,"
   - Step 2. From the entity list select "protein/complex/functionalclass," and from the relations list select "GeneticChange." Select "Next."
   - Step 3 Select "Finish."

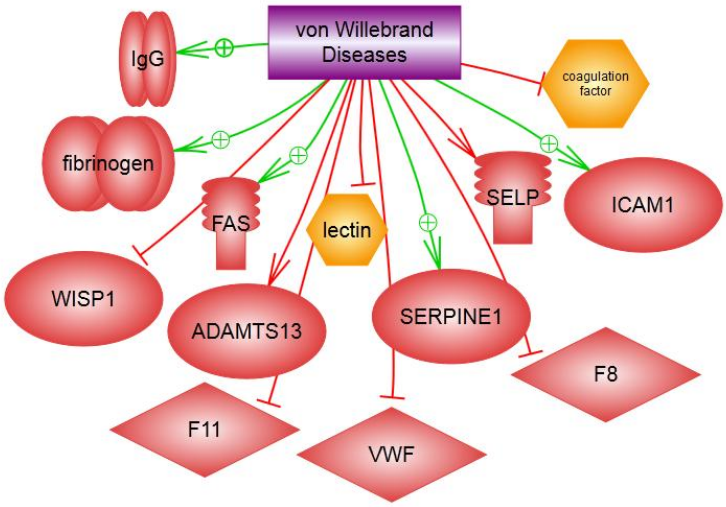


5. Which proteins are changed in abundance in a disease?

Mine the database for genes increased in expression or proteins increased in abundance with a specific disease

Steps to follow:

1. Identify the disease of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select "Build Pathway" and follow the wizard:
   - Step 1. Select algorithm "Expand Pathway"; Objects Directions: downstream (arrow to the right); then select "Next,"
   - Step 2. From the entity list select "protein/complex/functionalclass," and from the relations list select "QuantitativeChange." Select "Next."
   - Step 3 Select "Finish."
3. To go Style > Active Style Sheet > By Effect

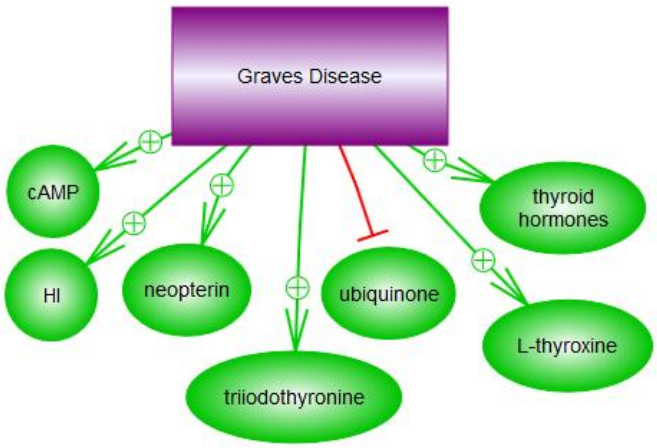


6. What metabolites are increased in a disease?

Mine the database for small molecules changed with a specific disease

Steps to follow:

1. Identify the disease of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select “Build Pathway” and follow the wizard:
   - Step 1. Select algorithm “Expand Pathway”; Objects Directions: downstream (arrow to the right); then select “Next,”
   - Step 2. From the entity list select “small molecule” and from the relations list select “QuantitativeChange.” Select “Next.”
   - Step 3 Select “Finish.”
3. To go Style > Active Style Sheet > By Effect

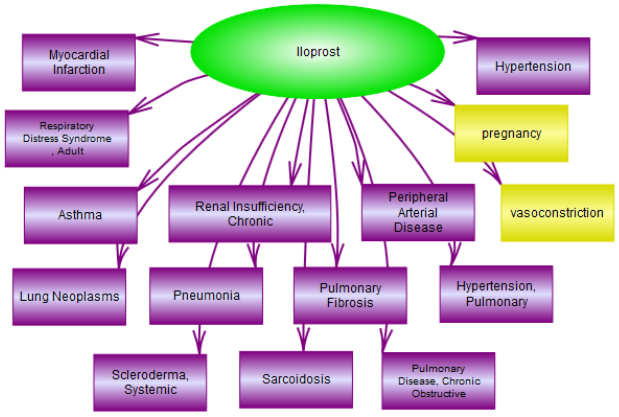


7. In which clinical trial(s) has a small molecule been tested?

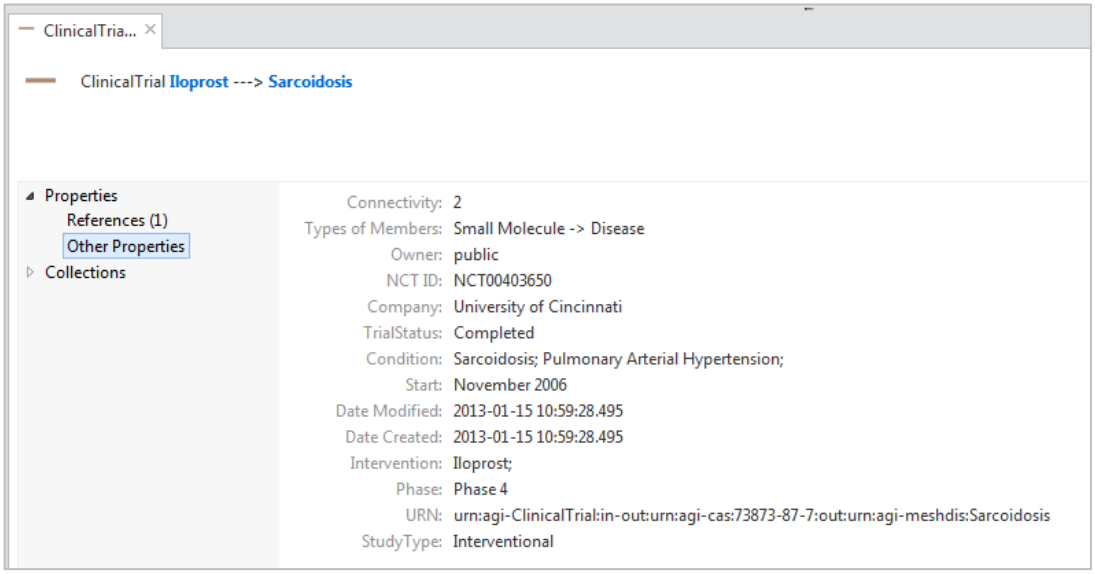
Mine the database for clinical trials associated with a particular small molecule.

Steps to follow:

1. Identify the small molecule of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select “Build Pathway” and follow the wizard:
   - Step 1. Select algorithm “Expand Pathway”; Objects Directions: downstream (arrow to the right); then select “Next,”
   - Step 2. From the entity list select “disease/cellprocess” and from the relations list select “ClinicalTrial.” Select “Next.”
   - Step 3 Select “Finish.”



The relation properties view provides additional information about the specific clinical trial, including study type, phase, trial status etc. Double-click on an individual relation to open the properties view. Select “Other Properties” to see detailed information about the clinical trial.



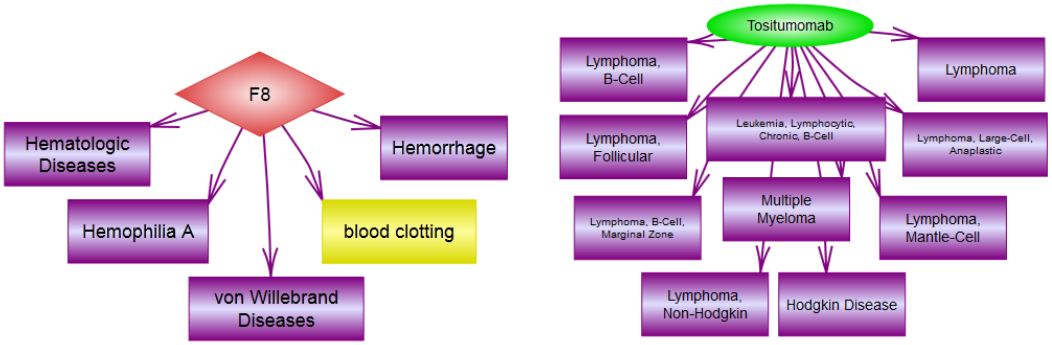
8. In which clinical trial(s) has a protein been tested?

Mine the database for clinical trials associated with a particular protein.

NOTE: In the ResNet database, monoclonal antibodies are actually represented as small molecules.

Steps to follow:

1. Identify the protein of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select "Build Pathway" and follow the wizard:
   - Step 1. Select algorithm "Expand Pathway"; Objects Directions: downstream (arrow to the right); then select "Next,"
   - Step 2. From the entity list select "disease/cellprocess" and from the relations list select "ClinicalTrial." Select "Next."
   - Step 3 Select "Finish."



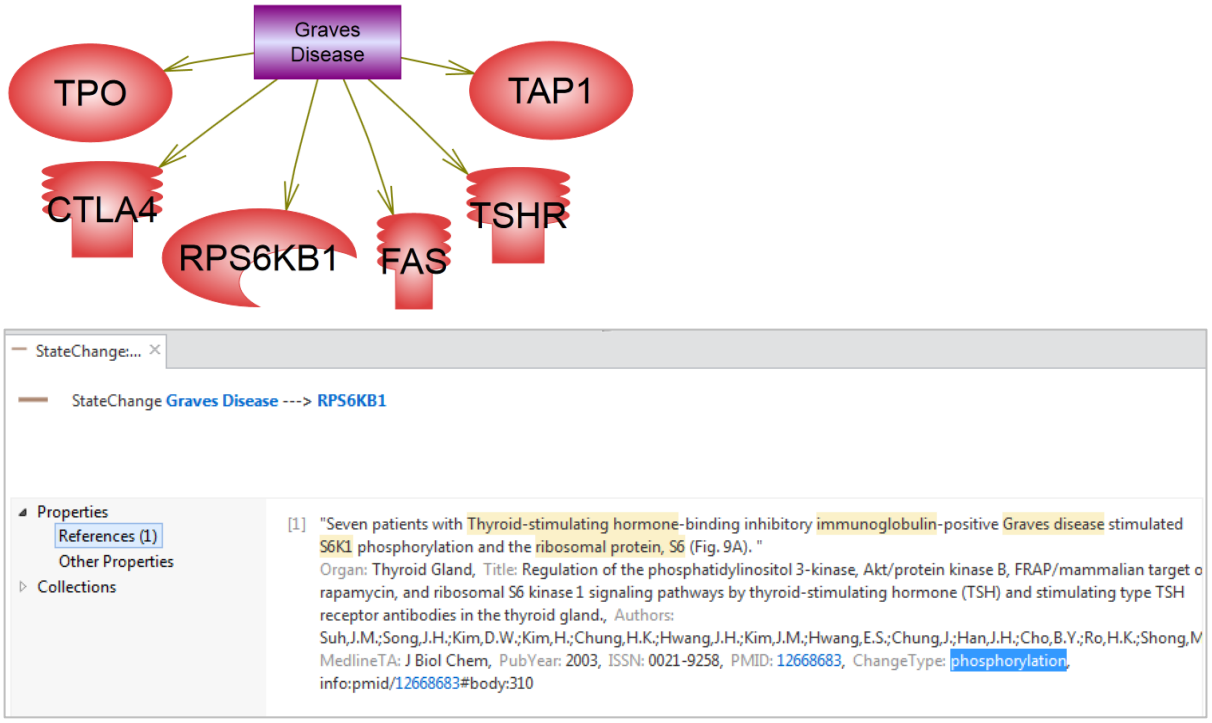
9. Are the activities of any proteins altered in a specific disease?

Mine the database for proteins with a StateChange relationship to a disease.

NOTE: State Change represents phosphorylation or alternative splicing events.

Steps to follow:

1. Identify the disease of interest in the database and add it to a new pathway view and select it.
2. From the Tools menu select "Build Pathway" and follow the wizard:
   - Step 1. Select algorithm "Expand Pathway"; Objects Directions: downstream (arrow to the right); then select "Next,"
   - Step 2. From the entity list select "proteins" and from the relations list select "StateChange." Select "Next."



Additional Information and Support

This Quick Start Guide to Building Pathways in ChemEffect and DiseaseFx describes some of the highlights of this database. If you have any questions about Pathway Studio please contact Customer Care:

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