Building A New Model

Models can be constructed using GRaPe in two ways.

- 1. Building from scratch
- 2. Importing an SBML document

Building from scratch

- Go to "File" -> New
- Type in the name of model then -> "OK"
- A new set of tabs appear (below)
- Use the "Species" tab to add, edit or remove species (metabolites and cofactors in the model)

| 4 | New Model | | 3 |
|---|----------------|-----------|---|
| | Create new mod | lel | |
| | Name | | |
| | | OK Cancel | |
| | | | |

- Use the "Enzyme" tab to add/edit/remove enzymes (proteins species) in the model
- The "Reaction" tab enables the construction of model reactions
- The "Option" tab provides methods for parameter estimation and also to save the model for simulation.

| ≝ GRaPe 1.0 | . – 🗙 |
|---|-------|
| File Help | |
| Start Page Help Guide Species Enzymes Reactions Options | |
| Add Species Details Name Concentration 0.0 Boundary Condition False True Add | |
| Edit Remove | |
| | |
| | |

Adding/Editing/Removing Species

 To Add a species; from the "Species" tab, enter in the species name, concentration and select its boundary condition then -> "Add". The boundary condition (BC) is a restriction imposed on a species specifying that its quantity is not determined by the set of reactions but by external constraints. When BC is set to true for a species, its concentration value remains the same as the value specified in the input data.

| Start Page File Help Guide Start Page Enzymes Reactions Options | _ | |
|---|---|--|
| Add Species Details Name Concentration Boundary Condition | List of Species Glucose [ID: s1 Conc: 5.7 Boundary Condition: true] | |
| Inputting Sowic File command cancelled by user Unable to open model. Check SBML version and va | ı lidity. | |

The newly added species is added to the model and displayed on the "List of Species" canvas

• To edit a species, select the species and click -> "Edit"

| ≝ GRaPe 1.0 File Help | | |
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| Start Page 📴 Help Guide | | |
| Species Enzymes Reactions Options Add Species Details | List of Species Glucose [ID: s1 Conc: 5.7 Boundary Condition: true] | |
| Boundary Condition False True Add | | |
| | Edit Remove | 1 |
| | | |
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| mporting Some File command cancelled by user Unable to open model, Check SBML version and va | ilidity. | Ĵ |

- After editing, click -> "Add" to add the species back to the list. NOTE: The species ID does not change after editing.
- **To remove a species;** select the species and click -> "Remove". The species is removed from the model and on-screen list.

Adding/Editing/Removing Enzymes

• From the "Enzymes" tab, choose whether to add the gene expression for the reaction or just the enzyme species (when the gene expression process is unknown).

| GRaPe 1.0 File Help | |
|---|---|
| Start Page Help Guide Species Enzymes Reaction Type List of Enzymes | |
| Cene Expression Enzyme Details Gene 0.0 mRNA 0.0 Enzyme 0.0 | |
| Edit Remove | |
| Importing SBML File command cancelled by user Unable to open model. Check SBML version and validity. | Ĵ |

- If only the enzyme is to be added. Simply select "Enzyme Only"
- Enter the enzyme's name and concentration and click -> "Add"

| CRaPe 1.0 GRaPe 1.0 Start Page Help Guide | |
|--|---|
| Species Enzymes Reactions Options Reaction Type Gene Expression Enzyme Details Name Conc Gene 0.0 mRNA 0.0 Enzyme 0.0 | List of Enzymes HK1 [ID: s2 Name: HK1 Conc: 0.0] |
| Importing SBML File command cancelled by dset Unable to open model. Check SBML version and v | Edit Remove |

• To enter the gene expression for a reaction

•

• Select "Gene Expression", enter the names and concentration amounts of the gene(s), mRNA and enzyme species then click-> "Add"

| Reaction Type O Enzyme Only Gene Expression | -List of Enzymes HK1 [ID: s2 Name: HK1 Conc: 0.0] e1 [Gene: [ID: s3 Name: g1 Conc: 0.0] mRNA: [ID: s4 Name: m1 Conc: 2.35] Enzyme: [ID |
|---|--|
| Enzyme Details Name Conc | |
| Gene 0.0 | |
| mRNA 0.0 | |
| Enzyme 0.0 | |
| Add | |
| | |
| | Edit Doman |
| | Edit Remove |

- To edit the enzyme or gene expression species, simply select the enzyme/gene expression species and click -> "Edit".
- Once editing is complete, click "Add" to add the enzyme/gene expression back.
- To remove an enzyme or gene expression species, select the enzyme/gene expression species and click ->"Remove".

| ≝ GRaPe 1.0 File Help | |
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| Start Page 📴 Help Guide | |
| Species Enzymes Reactions Options | |
| Reaction Type C Enzyme Only Gene Expression | List of Enzymes HK1 [ID: s2 Name: HK1 Conc: 0.0] e1 [Gene: [ID: s3 Name: g1 Conc: 0.0] mRNA: [ID: s4 Name: m1 Conc: 2.35] Enzyme: [ID: |
| Enzyme Details Name Conc Gene g1 0.0 mRNA m1 2.35 Enzyme e1 0.0 Add | |
| | Edit Remove |
| L Importing 35ML File command cancelled by user Unable to open model. Check SBML version and v | /alidity. |

Construction a reaction

From the "Reaction" tab, there are five (5) combo boxes. The first 2 allows for the addition of substrates and the last 2 enables the addition of products to a reaction. The middle one allows the user to select the enzyme/gene expression species for the reaction.

NOTE: A reaction MUST have an enzyme species/gene expression.

• The user must also specify the type of enzyme mechanism for the reaction. The default selection is "Random order" mechanism. (example below)

| 🕌 GRaPe 1.0 | |
|---|--|
| File Help | |
| Start Page Help Guide | |
| Reaction Details | Enzyme Mechanism HK1 P Q G6P ATP Compulsory Order Add |
| List of Reactions | Save Edit Remove |
| Importing Some File command cancelled by user Unable to open model. Check SBML version and validity. | |

• Once the substrates, products and enzymes have been selected using the combo boxes simply click -> "Add" to add the reaction to the model. (below)

| List of Reactions | HK1> 1 G6P + 1 ATP] [Rando | m Order1 | |
|----------------------------|-----------------------------|-----------|--------|
| | | | |
| | | | |
| | | | |
| | | | |
| Show Stoichiometric Matrix | | Save Edit | Remove |
| | | | |

- You can also specify the stoichiometric coefficient for each substrate or product in a reaction. The default value is 1.
- Once all reactions are added to the model, the user can view the stoichiometric matrix for the metabolic network by clicking on -> "Show Stoichiometric Matix" button. (An example is shown below).

| File | | | | | | | | | | | |
|-----------|-----------|------|-----------|--------|-------|------|-------|------|------|------|---|
| | Glu_Trans | НК | enzGlycog | en PGI | G3PDH | TPI | GAPDH | PGK | PGM | ENO | |
| Gluc_Out | -1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Glu_In | 1.0 | -1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| ATP | 0.0 | -1.0 | -1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | |
| G6P | 0.0 | 1.0 | -1.0 | -1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| ADP | 0.0 | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | -1.0 | 0.0 | 0.0 | |
| Glycogen | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| F6P | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| DHAP | 0.0 | 0.0 | 0.0 | 0.0 | -1.0 | -1.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| NADH | 0.0 | 0.0 | 0.0 | 0.0 | -1.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | _ |
| Glycerol | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| NAD | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | -1.0 | 0.0 | 0.0 | 0.0 | |
| GAP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | -1.0 | 0.0 | 0.0 | 0.0 | _ |
| BPG | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | -1.0 | 0.0 | 0.0 | _ |
| 3PGA | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | -1.0 | 0.0 | _ |
| 2PGA | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | -1.0 | _ |
| PEP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | |
| PYR | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| AcAld | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| CO2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| ETHANOL | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| F16bP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Trehalose | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| Succinate | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| t | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | _ |
| T: | 0.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |

Importing an SBML document

- Go to "File" -> "Open File"
- Select the file (in SBML) and click -> "Open"



- Again, the model appears as a list of tabs and provides functionalities for editing the model.
- The "Species" tab list all species in the model
- The "Enzyme" tab lists all enzymes (proteins species in the model)
- The "Reaction£ tab contains a list of all reactions
- The "Options" tab provides methods for parameter estimation

| art Page 📴 Full Glycolysis | |
|---|---|
| ecies Enzymes Reactions Option | S |
| Add Species Details Name Glycogen Concentration 0.0 Boundary Condition Galse True Add | List of Species • Gluc_Out [ID: s1 Conc: 50.0 Boundary Condition: true] Glu_In [ID: s2 Conc: 2.51 Boundary Condition: false] ATP [ID: s20 Conc: 2.51 Boundary Condition: false] G6P [ID: s3 Conc: 1.03 Boundary Condition: false] Gycogen [ID: s4 Conc: 0.04 Boundary Condition: false] GHAP [ID: s6 Conc: 0.11 Boundary Condition: false] DHAP [ID: s6 Conc: 0.14 Boundary Condition: false] GHAP [ID: s6 Conc: 0.14 Boundary Condition: false] DHAP [ID: s6 Conc: 0.14 Boundary Condition: false] DHAP [ID: s4 Conc: 0.04 Boundary Condition: false] GAP [ID: s24 Conc: 0.15 Boundary Condition: false] GAP [ID: s9 Conc: 0.03 Boundary Condition: false] BPG [ID: s11 Cone: 3.0E-4 Boundary Condition: false] Edit Remove |
| | |
| | |

- To edit a species/enzyme/reaction, simply select the entity and click -> "Edit".
- After editing, click on -> "Add" to add them back
- To remove a species/enzyme/reaction, simply select the appropriate entity and click ->
 "Remove". GRaPe allows for multiple deletion of species (metabolites), enzymes and
 reactions

Parameter Estimation

GRaPe provides two methods (genetic algorithm and Levengburg-Marquardt algorithm: GA-LMA) for estimating kinetic parameters in a model and another method for estimating the amount of proteins concentration needed by a reaction to reach a steady state (Steady State Enzyme Estimator (SSEE)).

Input Data for Parameter Estimation

All the estimation methods use the same input data. The data required for estimation is the flux value, substrates concentrations, products concentrations and enzyme concentration. **NOTE: The concentration enzyme MUST be a real number.**

For the SSEE, GRaPe uses the values in the last row of the input data as steady state values to estimate the proteins (enzyme) concentration.

For a Uni-Uni reversible reaction (both random and compulsory order) $A \leftarrow \rightarrow P$:

$$v = \frac{e_0(\frac{v_{+}^m a}{K_{mA}} - \frac{v_{-}^m p}{K_{mP}})}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}}}$$

where *a* is the concentration of substrate A, *p* is the concentration of product P. V_{+}^{m} is the rate of consumption of A (velocity of the forward reaction) and V_{-}^{m} is the rate of formation of P (velocity of the backward reaction); K_{mA} and K_{mP} are the Michaelis constant for A and P respectively; e_0 is the total concentration of the enzyme and *v* is the rate (or velocity) of reaction.

 $V_{+}^{m}/K_{mA} = K_A$ and $V_{-}^{m}/K_{mP} = K_p$ in a traditional reversible Michaelis-Menten rate equation.

The input data required by GRaPe to estimate the kinetic parameters for the reaction above should be in a tab-delimited format. The data can be prepared in Excel then copied into Notepad document and saved as a .txt file.

NOTE: GRaPe only matches the Ids in model to the ids in the input data. NOT THE NAMES OF SPECIES AND REACTIONS.

| v | а | р | e ₀ |
|--------|----|----|----------------|
| 121.48 | 50 | 12 | 1 |
| 121.48 | 50 | 12 | 1 |
| 121.48 | 50 | 12 | 1 |
| 121.48 | 50 | 12 | 1 |
| 121.48 | 50 | 12 | 1 |
| 121.48 | 50 | 12 | 1 |

From the "Options" tab

- Select the estimation method either SSEE or GA-LMA
- Choose the input data file then click -> "Run Estimation"

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|---|--------------------------------|--------------|-------------------|-----------------|
| ecies Enzymes Reactions | Options | | | |
| Paramter Estimation Method | | | | |
| | | | | |
| Steady State Enzyme Estimation | ator 🛛 🖲 Genetic Algorithm (GA | -LMA) Choose | e Data File Run E | stimation |
| | | | | |
| Results | | | | |
| Cours | | | | |
| kmP: 9580078.125 | | | | <u>^</u> |
| [v2 HK] [1 Glu In + 1 A] | ГР <> 1 | ו מתגו + הפס | Pandom Orderl | |
| 0bj Function: 2.631155737733 | | oor timorj [| Kandom Orderj | |
| V+: 125.0, | | | | |
| V-: 9.21875E-5, | | | | |
| kiA: 7.666015625E-9, | | | | |
| kiB: 0.006845703125, kiQ: 4.99999999999999996E-6, | | | | |
| kiQ: 4.99999999999999999990E-6, kiP: 8.681640624999999E-6, | | | | |
| kmB: 1, | | | | |
| kmP: 1 | | | | |
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| | | | | |
| | | | | Convert 2 SBML |
| , | | | | CONVERT 2 SDIIL |

• Once estimation is complete, click -> "Convert 2 SBML" to save the model.