**First metabolic reconstruction and constraint-based models of *B. malayi* which can be used to identify novel drug targets**

**Research article:** Modeling the metabolic interplay between a parasitic worm and its bacterial endosymbiont allows the identification of novel drug targets (2020) *eLife 9:e51850*

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**| New findings or achievements of this research paper (in one sentence)**

Novel therapeutic targets can be found by modeling the metabolic interaction between a *B. malayi* and its bacterial endosymbiont.

**| Goals of this research**

* Goal1: Researchers present a new way to solve the lymphatic filariasis problem caused by filarial nematode *Brugia malayi* (not to be bitten by mosquitoes is the only way to solve the problem so far).
* Goal 2: Research *Wolbachia* as a target.
* Goal 3: Researchers’ goal was to make the first compartmentalized *B. malayi* metabolic pathway model which is *i*DC625.
* Goal 4: Researchers tried to find how *B. malayi* adapt to different environments with metabolic pathways and predict essential reactions for survival of *B. malayi*.
* Goal 5: Our model is the first to pathways the anaerobic metabolic, and accurate predictions as low oxygen environments are biologically relevant for parasitic nematodes.

**| Key hypothesis of this research**

* Hypothesis 1 : The *i*DC625 model is the first genome scale metabolic model for *B. malayi*. This model is used to show the network and metabolic pathways of this parasite.
* Hypothesis 2 : The presence of *Wolbachia* directly impacts model dynamics, both through the production of metabolites that contribute to the biomass objective function.
* Hypothesis 3 : *Wolbachia* will affect *B. malayi* depending on different conditions.
* Hypothesis 4 : Life stage specific metabolic models of *B. malayi* reveal a dynamic reliance on alternative pathways.
* Hypothesis 5 : The model was able to identify a set of essential reactions which represents potential targets for developing new treatment against *B. malayi*.
* Hypothesis 6 :The researchers used three drugs to test and validate the performance of their metabolic model by assessing their effects on *B. malayi*.

**| Information of mathematical model & machine learning strategy**

* Biological network model using linear problem solving
* Input: Metabolic pathway data
* Output: optimal flux values
* Parameter estimation method: Parameters were set by the values that article explained.
* Model training and validation: FBA(flux balance analysis)

**| Results of team project**

* Introduction video: <https://www.youtube.com/watch?v=A5fFyY2ptyg>
* Final result video: <https://youtu.be/AlCZJRUCR-8>
* Presentation slide: [Paper Summary](https://docs.google.com/presentation/d/1WHijkE_VWK3lq1-ATbM0w6_nUk44Cp1JkIxgdk2ajVo/edit?usp=sharing)

**| Computer codes used for this project**

**Codes that your team wrote**

* Colab file URLs
  + <https://drive.google.com/file/d/10FGBmjw8WMweuQTXUd07bm2Jjs3ki7x0/view?usp=sharing>
* Google Drive URLs
  + <https://drive.google.com/drive/folders/1e-sXmLETMTFkk0pLpfz5C1mRCp4wejmc?usp=sharing>

**Codes borrowed from the paper or other sources**

* Journal website
  + <https://elifesciences.org/articles/51850>
* Public resources
  + <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA557263>
* Github
  + <https://github.com/opencobra/m_model_collection/tree/master>
  + <https://github.com/ThierryMondeel/Systems_Biology_FBA_tutorial>

**| Original and reproduced figures**

|  | **Original figures in the paper** | **Figures reproduced by your team** |
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| **1** |  | **Figure made through Cytoscape**    **Figure made through python (NetworkX package)** |
| **2** |  | **A model was created using the same data. But the figure of L3 did not come out.**    **When only L3 was pulled out individually, the value was printed on the model.** |
| **3** |  |  |
| **4** |  |  |
| **5** |  |  |

**| Step-by-step procedure of making figures: Figure 1**

Please provide a detailed description sufficient for your friends to reproduce the figure.

| **Figure 1 made through Cytoscape**   * **Step 1: Downloading data from here (**[**https://github.com/ParkinsonLab/Brugia\_metabolic\_network**](https://github.com/ParkinsonLab/Brugia_metabolic_network)**). The file name is model\_bm\_6.sbml in ‘model’ file.** * **Step 2: Download Cytoscape in** [**https://cytoscape.org/**](https://cytoscape.org/)**. It is open source so it is free to download.**      * **Step 3: Open Cytoscape. And, import the model to Cytoscape.**        * **Step 4: Click ‘layout’ and choose ‘Apply Preferred Layout’. We choose ‘Apply Preferred Layout’ because we tried with different tools and layout name was not specified in the article. (Article used ‘Pathway Tools but it is available for universities or government institution that has license. )**        * **Step 5: Go to ‘filter’ and then, choose ‘column filter’.**      * **Step 6: Choose ‘Node: sbml compartment’. Figure 1 was drawn with each compartment which were cytosol, mitochondria, and *Wolbachia*. After choosing ‘Node: sbml compartment’, filter the node by “ ‘Node: sbml compartment’ contains c”. This means we are going to filter nodes that are the compartment of cytosol. For other compartments, you can type ‘m’ for mitochondria and ‘w’ for *Wolbachia* instead of ‘c’.**        * **Step 7: After filtering the node with compartment, you can see the yellow nodes that are filtered. For this picture, yellow nodes mean metabolites which compartment is cytosol.**        * **Step 8: Repeat step 6 to step 8 for mitochondria and *Wolbachia* compartment.**   **Figure 1 made through python code and libraries**   * **Step 1: Downloading data from here(https://github.com/ParkinsonLab/Brugia\_metabolic\_network)** * **Step 2: Open Google Colab to plot figure with python code.** * **Step 3: To plot figure 1 with python, we have to import some libraries and packages. matplotlib.pyplot is for plotting data. networkx is for network analyzing and drawing. libsbml is for reading sbml file.**      * **Step 4: Using the libsbml package, we have to bring and read a sbml model file. We can use libsbml.readSBML() to read a sbml file. Then, save this to a model by document.getModel().**      * **Step 5: We first have to define a graph to draw a graph.**      * **Step 6: First, we have to add metabolites to a graph. This is a code based on the ‘libsbml’ package to find metabolites. And, since figure 1 is drawn with each compartment, we have to find a metabolite that are in specific compartment.**     **In this case, we are going to find a metabolites (nodes) that are in cytosol as ‘c’. For mitochondria, we can replace it with ‘m’ and for *Wolbachia*, we can replace with ‘w’.**     * **Step 7: Now, we have to add a reaction that connects nodes for edges. This is a code based on the ‘libsbml’ package to find reactions that connect nodes found from above code. This code works by a node of reactant and product connected each other to make a network.**      * **Step 8: We used kamada kawai layout. We tried with other layouts but this layout was mostly similar with actual figure 1 in the article. (Specific layout was not explained in the article.)**      * **Step 10: Then, we can plot nodes and edges to show the metabolic network in each compartments (cytosol, mitochondria, and *Wolbachia*).**      * **Step 11: Repeat 6 to 10 for other compartment which are mitochondria as ‘m’ and *Wolbachia* as ‘w’.** |
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**| Step-by-step procedure of making figures: Figure 2**

| * **Step 1: Downloading data from here (**[**https://github.com/ParkinsonLab/Brugia\_metabolic\_network/tree/master/models**](https://github.com/ParkinsonLab/Brugia_metabolic_network/tree/master/models) **, All the excel files except for the ‘model\_bm\_6.sbml’ file.)** * **Step 2: Follow the steps in** [**https://cobrapy.readthedocs.io/en/latest/index.html**](https://cobrapy.readthedocs.io/en/latest/index.html) **to understand the cobra tools using.**      * **Step 3: Installed from Colab to use cobra toolbox.**      * **Step 4: Let’s create a simple toy model to see if the FBA model is created.**      * **Step 5: If you have confirmed that it is running normally, actually reconstruct the model. In order to do so, we bring in the necessary data. In the process of loading the excel file for the first time, we had difficulty because the total number of metabolism and reaction in the file was read as 0.**     **So we read the excel file using import\_excel\_model. Items from 'MET\_SHEET\_ID' to 'DEFAULT\_OBJECTIVE\_COEFF' in the image file were filled with the column name in the Excel file.**     * **Step 6: Import excel files of a total of 10 life stages(open~M120) using the import\_excel\_model function.**      * **Step 7: Check whether the data of all files has been loaded successfully.**        * **Step 8: Since figure2 draws a graph to see the effect of the *Wolbachia* weight on the maximum objective function flux in 4 different nutritional conditions, the code to set the lower and upper bounds of glucose and oxygen differently and the lower and upper bounds of biomass are the thesis Configure the function to do the same as set in.**      * **Step 9: Set the values of the four nutritional conditions differently, the same as the nutritional conditions presented in the paper, and open the ‘Open’ model first.**          * **Step 10: A total of 10 models were printed separately.**            * **Step 11: Use subploe() to view 10 models at a glance.** |
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**| Step-by-step procedure of making figures: Figure 3**

| * **Step 1: Downloading data from here (**[**https://github.com/ParkinsonLab/Brugia\_metabolic\_network**](https://github.com/ParkinsonLab/Brugia_metabolic_network)**).**   **All model files are required.**     * **Step 2: Except one sbml file format, all of the model files are excel files. We need to read the excel file through the import\_excel\_model function. (COBRApy doesn’t have a read\_excel model function…)**      * **Step 3: Import models from your own file path and save data in each variable that has its own model name. (The picture below is an example)**      * **Step 4: Write Heatmap function code in python.**     **While writing this code, there are several steps to follow.**   1. **Set the range of oxygen and glucose levels.** 2. **Perform FBA for all combinations of oxygen and glucose.** 3. **Find each reactions we are going to reproduce through the heatmap**   **In this case, we found five reactions which have ids of [R01082\_M, R00253, SINK\_3, SINK\_2, SINK\_4] and objective value.**   1. **Set the color map which would be used in this heatmap.** 2. **Draw the heatmap through matplotlib library in python.**  * **Step 5: If you implement heatmap(model name) function, you can reproduce one row(line) of the original figure.**      * **Step 6: If you implement the heatmap function multiple times, you can reproduce all the heatmaps in various models like the picture below.** |
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**| Step-by-step procedure of making figures: Figure 5**

| * **Step 1: Downloading data from here (**[**https://github.com/ParkinsonLab/Brugia\_metabolic\_network**](https://github.com/ParkinsonLab/Brugia_metabolic_network)**).**   **All model files are required.**     * **Step 2: Except one sbml file format, all of the model files are excel files. We need to read the excel file through the import\_excel\_model function. (COBRApy doesn’t have a read\_excel model function…)**      * **Step 3: Import models from your own file path and save data in each variable that has its own model name. (The picture below is an example)**      * **Step 4: Set *Wolbachia* weights and create an empty objective flux list.**      * **Step 5: Set the lower bound and upper bound of glucose and oxygen according to the model.**      * **Step 6: Calculate objective value through model.optimize function.**      * **Step 7: We can draw figure 4 by simple python code, which has x-axis of *B. malayi* life stage model and objective function flux units of y-axis for every four conditions(HOHG, HOLG, LOHG, LOLG). The picture below is an example figure in HOHG condition.** |
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**| Step-by-step procedure of making figures: Figure 6**

| * **Step 1: Downloading data from here (**[**https://github.com/ParkinsonLab/Brugia\_metabolic\_network**](https://github.com/ParkinsonLab/Brugia_metabolic_network)**).**   **All model files are required.**     * **Step 2: Except one sbml file format, all of the model files are excel files. We need to read the excel file through the import\_excel\_model function. (COBRApy doesn’t have a read\_excel model function…)**      * **Step 3: Import models from your own file path and save data in each variable that has its own model name. (The picture below is an example)**      * **Step 4: Set the lower bound and upper bound of glucose and oxygen according to the model.**      * **Step 5: Calculate objective value through model.optimize function.**      * **Step 6: Define count\_reactions\_with\_flux function to count ‘real’ reactions that do not have flux value of ‘0’.**     **You can set the compartment in this function to find out the number of reactions for each compartment. For example, in the above code, metabolite.compartment == ‘c’ means we only want to see the ‘cytosol’ compartment. ‘m’ indicates mitochondria, and ‘w’ indicates *Wolbachia*.**   * **Step 7: Implement the count\_reaction\_with\_flux function with each model and save them.**     **Then, the code result would be like the picture below, showing the number of reactions by each compartments.**   * **Step 8: We could not reproduce the figure 6 in a perfect way, so it would not be possible to explain and proceed more steps.** |
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**Conclusions**

Write the conclusion of your team project

| List what your team has done successfully | Things your team didn't succeed |
| --- | --- |
| * We produced some figures from the data given in the article. * Successfully used sbml file with different tools like Cytoscape and python (libsbml package and Cobra package). * Procedures for producing figures were not clearly defined but we figured out how to use data and plot them with a research of different methods. * We were able to read and understand sbml file successfully. So, we could able to use them with code and get values that we want from a sbml file. * We were successfully used flux balance analysis with using python package called ‘Cobra’. | * This article used a tool called ‘Pathway Tools’. However, this tool is available for universities and government institutions that have licenses. Thus, using different tools can be a thing that we did not success. * In figure 1, the layout name was not specified in the article. So, figure 1 in the article and figure 1 that we reproduced looks different. * Figure 1 in the article has a essential reactions that nodes are colored. However, we could not color them because we could not find essential reactions defined in the file. So, we only plotted a metabolites of each compartment and reactions. * We tried to reproduce the same results as in Figure 2, but the condition constrain was specified differently according to the four nutritional conditions, and the lower bound and upper bound values of biomass were also specified as specified in the paper, but the graph did not come out the same. As a result of outputting the value separately, it was often output as a value of 0, but I could not find the reason. When this failed, the solution was not presented in the paper, so even when the biomass, lower bound, and upper bound values were specified differently, the same results as figure2 did not come out. * When we tried to reproduce Figure 6, we did not know how to set the code for parsimonious solution of the reactions exactly. * Figure 7(the effect of drug target) could not be reproduced due to the lack of in vivo data and out unskilled usage of cytoscape libraries. * We sent an email to the original author of the paper and asked if we could get the code needed to make the figure in the article, but we didn't get an answer. (so we developed the code by ourselves) |

**Meetings of Team 2 for Final Report**

