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Pathway Pioneer: A Web-Based Metabolic Network Layout Extension

Harsh Dosi
Utah State University

PATHWAY PIONEER: A WEB-BASED METABOLIC NETWORK LAYOUT EXTENSION

by

Harsh Dosi

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Approved:

Dr. Nicholas Flann Major Professor	Dr. Kyumin Lee Committee Member
Dr. Tung Nguyen Committee Member	Dr. Mark R. McLellan Vice President for Research and Dean of the School of Graduate Studies

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Logan, Utah

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ABSTRACT

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Harsh Dosi, Master of Science

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Major Professor: Dr. Nicholas Flann
Department: Computer Science

The number and complexity of genome-scale metabolic networks is increasing as new systems are characterized and existing models are extended. Tools for visualization of network topology and dynamics are not keeping pace and are becoming a bottleneck for advancement. Specifically, visualization tools are not optimized for human comprehension and often produce layouts where important interactions and inherent organization are not apparent. Researchers seek visualizations in which the network is partitioned into functional modules and compartments, arranged in linear, cyclic, or branching schema as appropriate, and most importantly, can be customized to their needs and shared. Challenges include the wide diversity in the biological standards, layout schemas, and network formats. This work introduces a web-based tool that provides this functionality as an extension to the existing web-based tool called Pathway Pioneer (www.pathwaypioneer.org). Pathway Pioneer is a dynamic web-based system built as a front-end graphical user interface to the flux balance analysis tool COBRA-py. Full click-and-drag layout editing capabilities are added allowing each metabolite and reaction to be translated and rotated as connecting edges are automatically redrawn. Initial automated layouts for new models maximize planarity while clustering reactions based on subsystem module and compartment. The users are given maximum flexibility to design specific layouts while details of convention, such as joined

in and outflow of reaction edges, disconnected co-factors, and connected metabolites, are automatically handled. Layouts can be shared among researchers and explored to archival Symphony format, along with pdf and png images. This tool provides the user with a semi automatic layout algorithm along with graphical and interactive tools to fully customize the network layout for optimal comprehension. Export capabilities are compatible with COBRA-py and other visualization tools. It provides a platform for share model development and innovation to the community, sharpening the R&D curve, and improving the turn-around time of model reconstruction at the genome-scale. Pathway Pioneer provides unique capabilities in customization of metabolic networks that complements and overcomes limitations of the growing body of existing tools.

(31 pages)

PUBLIC ABSTRACT

Pathway Pioneer: A Web-Based Metabolic Network Layout Extension

HARSH DOSI

The number and complexity of genome-scale metabolic networks have seen a rapid growth in the past 10 years. Today, more than 450 genome-scale metabolic reconstructions of biological systems are characterized and existing models are extended. With the growing influence of these reconstructions on biomedical and biological research, the field has observed a shift from an inward focus on method development to an outward focus on application development.

Static state analysis of metabolic networks in textual format is too cumbersome and time consuming. In addition, visualization and arrangement of these networks to facilitate their analysis is an open research problem. A visually intuitive laid out metabolic network facilitates its analysis for pathway search and metabolic engineering. Currently existing layout algorithms are limited and cannot produce an intuitive layout for a metabolic network. Also, the layouts produced by automatic algorithms suffer from a hair ball layout problem.

Pathway Pioneer Layout extension presents a solution to this problem by providing a graphical user interface-based tool to customize and edit the network according to the user's choice. Existing layouts can be modified using simple drag and drop functionality resulting in metabolite and reactions to place anywhere on the network. Connecting edges are automatically drawn on these maneuvering of reactions and metabolites.

Pathway Pioneer also allows the user with a versioning system to provide backup and branching of the development work. Sharing of layouts with extensive import and export capabilities provides a rapid development platform for metabolic engineering.

Pathway Pioneer layout extension combined with its analysis tools presents a complete suite for metabolic network visualization and analysis.

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CHAPTER 1

INTRODUCTION

Pathway Pioneer is a dynamic, clickable, browser-based visualization and analysis system for metabolic network models retrieved from databases such as BiGG or developed in-house as SBML or XLS compliant files. The user can customize the network layout to visually organize the metabolites and reactions into functional modules. The tool supports zooming and panning, level-of-detail control, flux visualization, keyword searching, and hierarchical subsystem organization. During analysis, a reaction may be knocked out, set as an objective, looked up in a database or many other operations by a single click on the visualized network. Following each operation the visualization is refreshed with the new metabolic flux values. The system supports model revision control to manage alternative network configurations and sharing of models and layouts to the broader community. Since the tool is web-based, the computationally intensive model analysis overhead is transferred from the user computer to remote servers, enabling the application of high performance cloud-based resources for greater efficiency and scalability. Although, Pathway Pioneer supports Flux Balance Analysis (FBA), visualization and customization of metabolic networks. The focus of this thesis is the customization of network layouts.

CHAPTER 2

BACKGROUND

Visualization of scientific models must strike a balance between convention and innovation in their user interaction. Convention is needed to provide a shared interpretation and to support collaboration along with transfer of knowledge among researchers. Innovation enables users to develop new ways of organizing the models as they grow in complexity and as new applications arise. No more is the requirement true than in the visualization of genome-scale metabolic network models that are developed at an increasing rate for bacterial, archaeal and eukaryotic systems, each encompassing thousands of interconnected reactions, evidenced in the Kyoto Encyclopedia of Genes and Genomes (KEGG) [1], EcoCyc [2], BioCyc [3], and metaTIGER [4] databases. Metabolic networks are characterized as a highly complex network with metabolites as nodes and enzyme catalysed reactions as edges. These networks encode abstract knowledge of cellular metabolism including interactions between reactants and enzymes, inter and intra subsystem flux flows, organization into sub systems and linearly organized pathways.

Metabolic networks are characteristically defined as complex [5] and scale free graphs [6]. The principle challenge is to visually organize such networks as hyper-graphs so as to facilitate knowledge retrieval to elucidate the phenotypes and genotypes of the species and the complex relationship among different components of the metabolic network. Jeong et al. [7] shows that information extraction from these networks depends upon how these networks are visually presented. Many approaches to layout these metabolic networks have been proposed. Guimera and Nunes Amaral [8] and Ravasz et al. [9] proposed layout algorithms that takes into account the spatially or chemically isolated functional modules of metabolic networks composed of several cellular and specific objective components, considered as building blocks of cellular organization [10, 11].

Automated layout algorithms of these hyper graphs can be categorized in two approaches: hierarchical and force directed layout methods. Force directed algorithms are described in [12–14] and are used in many visualization tools like Cytoscape [15] and Proviz [16]. They produce layouts unsuited to the visual requirements described for metabolic networks. Hierarchical layout algorithms (also known as layered graph drawing algorithms) are optimized for acyclic graphs but metabolic networks are mostly cyclic. This approach arranges the vertices of the graph in horizontal lines, and edges connecting the different layers, achieving downward planarity. Pathfinder tool [17] embeds these algorithms in their tool. The layout generated using these automatic algorithms can be counter intuitive and messy that prevents the user from extracting knowledge from these networks.

Figure 2.1 (b-d) shows the layouts of the same metabolic network produced by existing layout algorithms embedded in Cytoscape, while Figure 2.1 (a) is laid out manually using a convention, frequently used by researchers. It is clearly seen that even in a small network such as shown here, automatic layout algorithms do not produce comprehensible layouts. While the time needed to produce an automatic layout is much less than manually laying out the network, manual networks are much more useful.

In addition to visual display, visualization tools have to manage with poorly defined and diverse standards of network formats for importing and exporting layouts. Various network standards described in [18] exist in the literature, but are incompatible. Novere et al. [19] defines as accepted standard of visual notation for network diagrams using a comprehensive set of symbols with precise semantics, together with detailed syntactic rules regarding the construction and interpretation of maps. Finney and Hucka developed the SBML [20] format that as a standard format for metabolic and other networks follows and currently used by 89 software worldwide. Pathway Pioneer enables model import and export in SBML format.

Another challenge includes loose coordination between different biological communities, resulting in heterogeneous, redundant and slower research in this field. Most of the current visualization tools are stand alone and are also incompatible with each other. In addition,

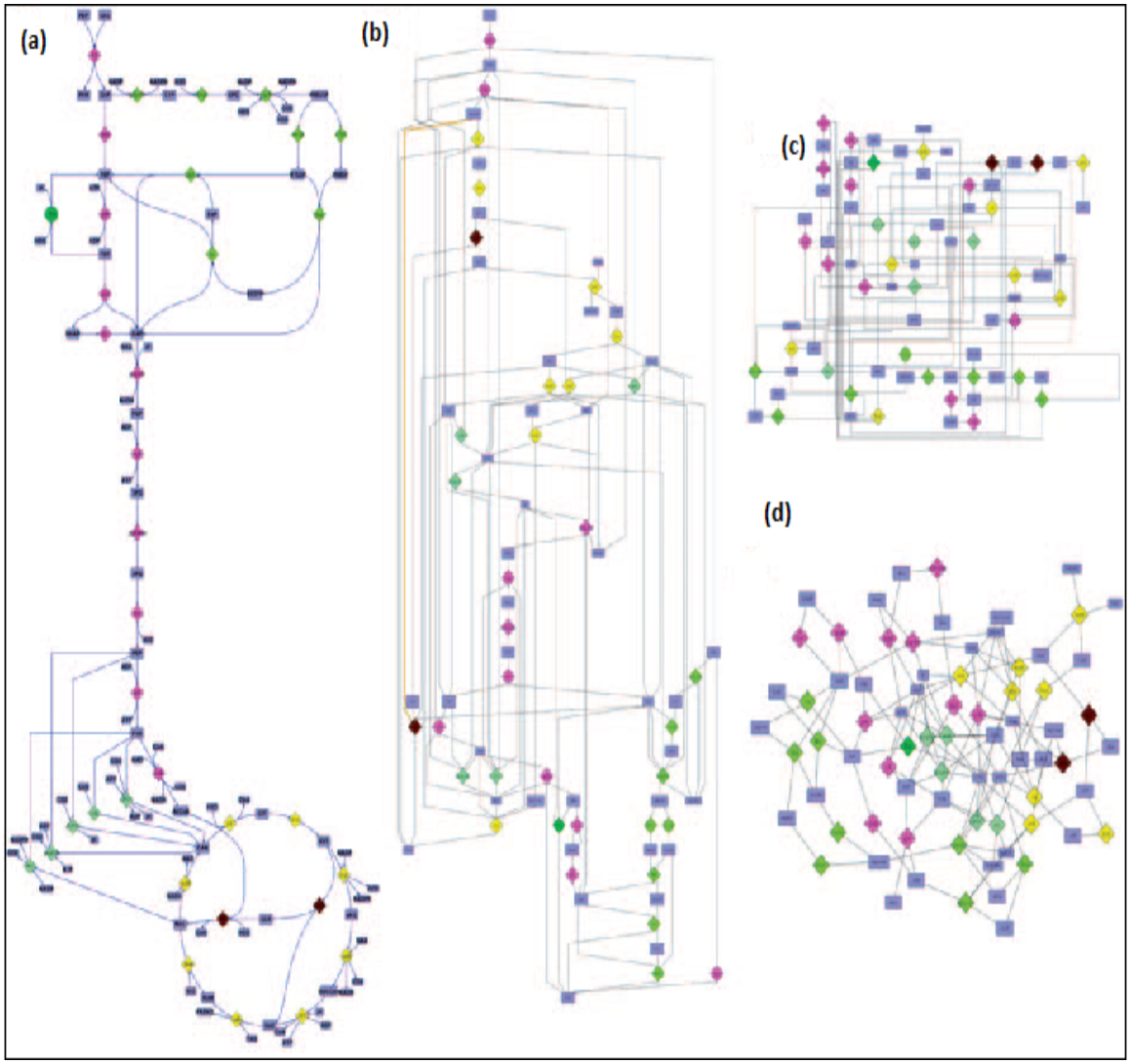

Figure 2.1: Layout of central metabolic pathways: (a) manually drawn pathway (b-d) layout created using several algorithms provided in Cytoscape [15].

none provide a feature to export the layout information of the network so as to use it with other tools or the community as a whole. The solution to this challenge lies in the sharing of knowledge and providing a common platform to all the scientists and researchers working in this field. Pathway Pioneer embraces the concept of "share and expand" by being a web based platform and also provides capabilities to publish newly developed models and share network layouts in different formats.

CHAPTER 3

SYSTEM OVERVIEW

Pathway Pioneer (PP) is built as a web based system using Model-View-Controller (MVC) architecture. It holds a three-tier architecture with two major domain of applications. First is the network analysis tool and second is the network editing and customization tool. Figure 3.1 gives a brief tour of www.pathwaypioneer.org.

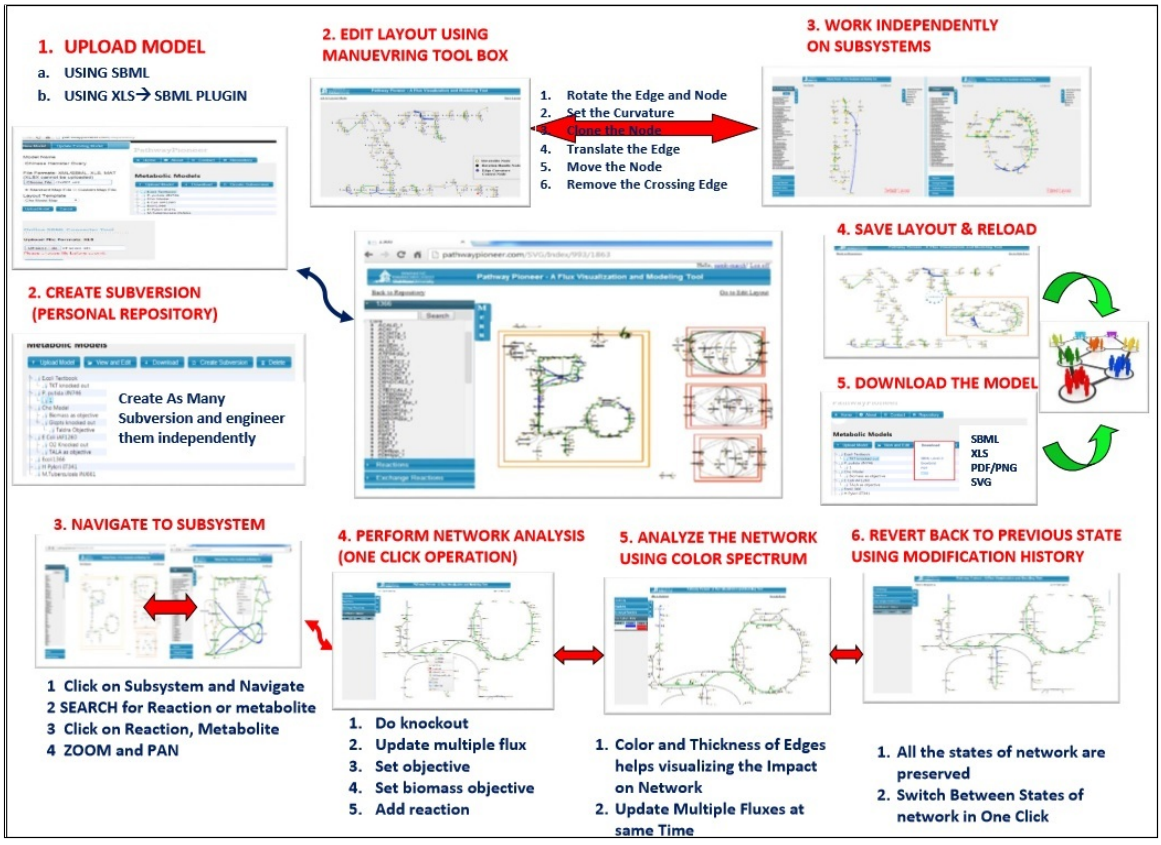

Figure 3.1: Image providing a overview of Pathway Pioneer. It illustrates the various functionalities and a general work flow in Pathway Pioneer using these functionalities.

Pathway Pioneer is powerful graphical interface for the COBRA Toolbox [21,22], which

is well known for Genome Scale Metabolic Reconstruction. To save and share the layouts PP creates map files which were initially made available by KEGG database [1] but were later discontinued.

Pathway Pioneer layout tools combine convention and innovation to produce highly intuitive and understandable visualizations of reconstructed networks that can be easily extended and modified. All layouts produced by PP incorporate the following conventions: a) Metabolites are nodes, reactions are straight edges. b) All input metabolites of a reaction are connected to an end of the reaction line. Similarly for the output metabolites. c) The edges connecting a metabolite to a reaction are drawn as a smooth line incident to the reaction line. Innovation is allowed in the placement and rotation of any reaction and metabolite and also in whether a metabolite common to two reaction is drawn as a single or duplicate node.

The next section contains the structural layout editing capability of PP to create high utility layouts of metabolic networks.

3.1 Core Functionalities

Figure 3.2 shows the haphazard and messy layout generated by automated layout algorithm from the Matlab Bioinformatics Toolbox used by the Paint4Net [23] tool. Contrary to this, Figure 3.3 shows a visually intuitive Citric Acid Cycle of denovo synthesized Chinese Hamster Ovary (CHO) model which is laid out using simple, interactive yet powerful layout tool box provided in Pathway Pioneer. To produce this layout, the user goes to the editing workspace of PP (by clicking the Edit Layout link, located in header). The editing workspace provides a set of graph maneuvering tools to rearrange metabolite nodes, reaction edges and eliminating the crossing reaction edge concerned in metabolic networks.

Pathway Pioneer decomposes the layout problem into subsystems. As shown in Figure 3.3, a particular subsystem of interest has to be selected and magnified, so that each metabolite and reaction in that section is clearly visible and also to provide with smooth dragging. PP provides the user with graphical maneuvering tools that enable the user to create any possible layout that follows the conventions mentioned earlier using standard UI

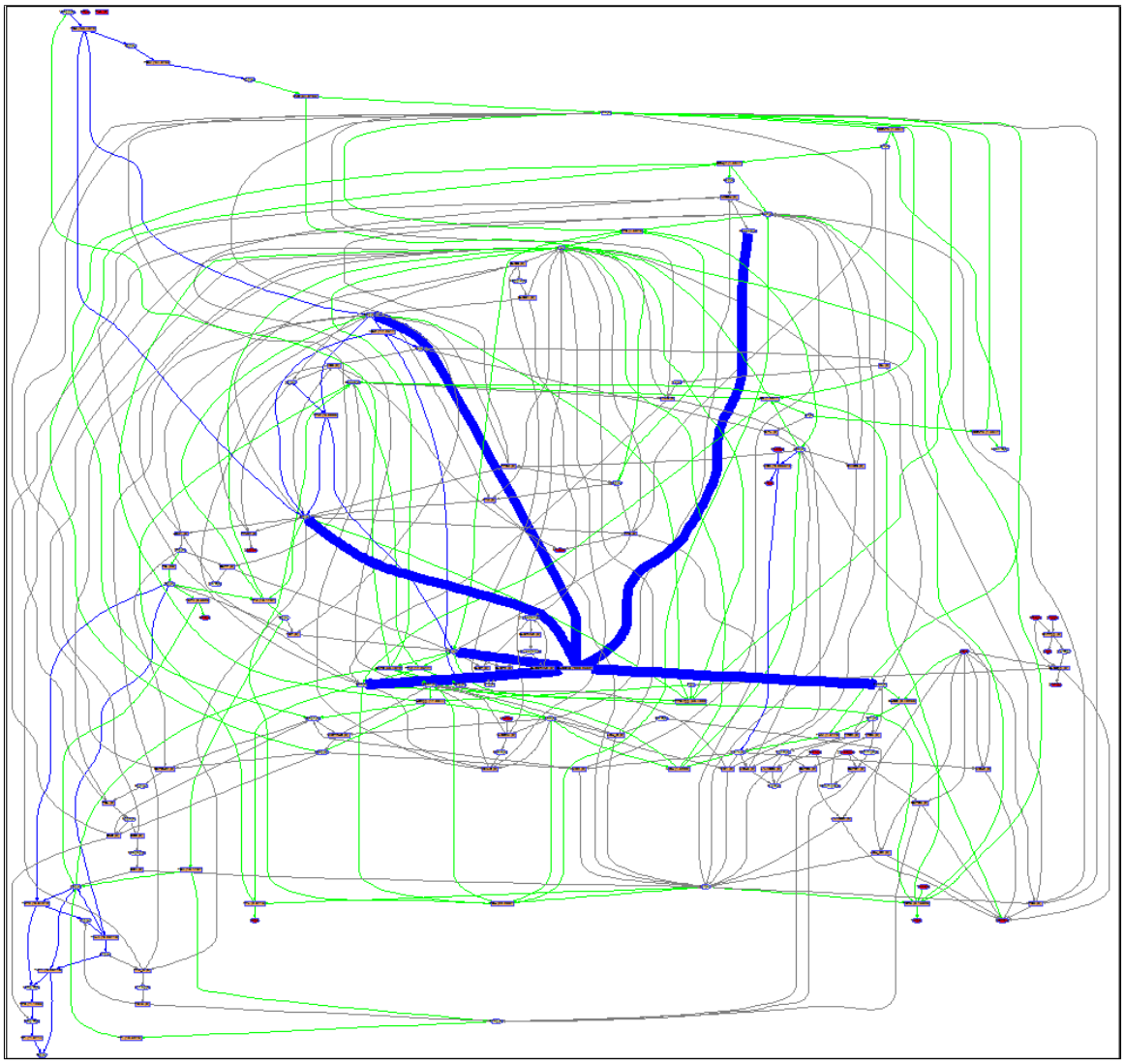

Figure 3.2: The Core metabolic network for *Escherichia coli 1366* cell visualized in Paint4Net [23]. The lack of organization and numerous line crossings make the figure unintelligible.

Drag-Drop-Switch operations. The following network editing operations are available:

Graph Maneuvering Tools: Pathway Pioneer provides a wide set of network maneuvering tools that enable the user to rearrange the position and rotation of any network component, While the user can rearrange reactions and nodes, certain aspects of the layout are handled automatically. Specifically, the system maintains alignment constraint that constrains the incident angle of incoming and outgoing metabolite-

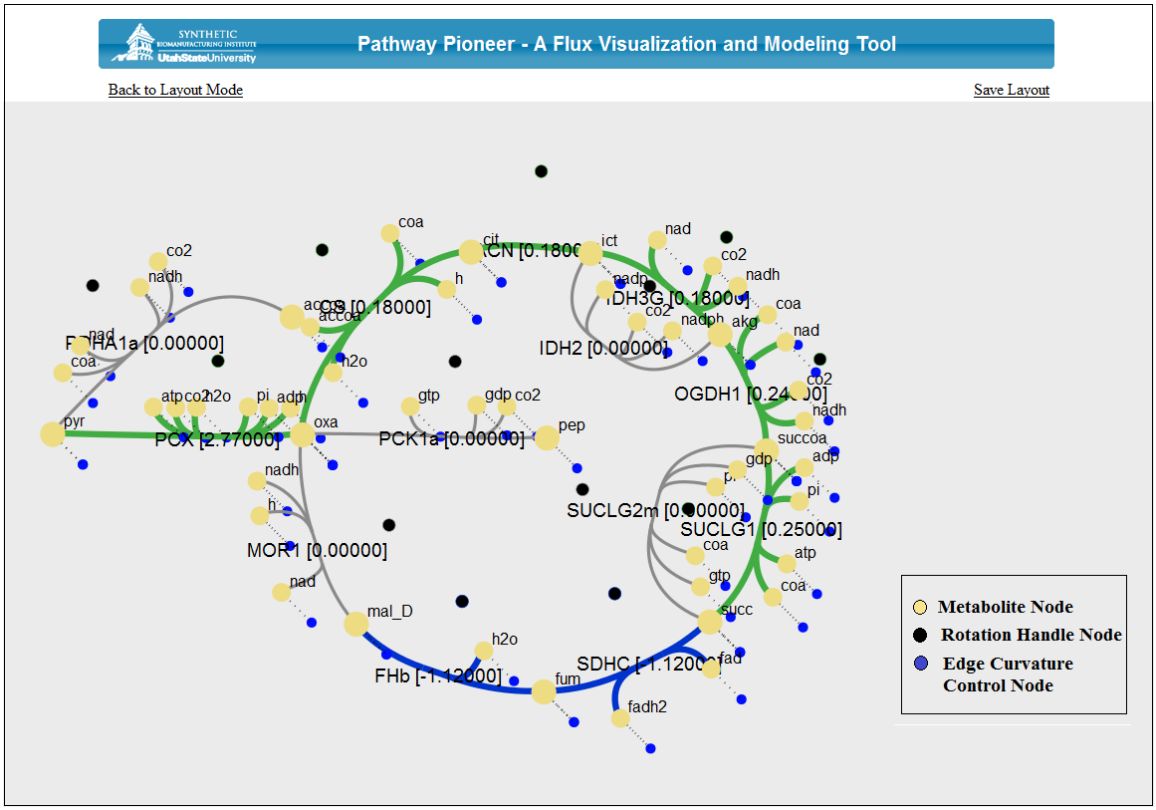

Figure 3.3: Pathway Pioneer: The web interface is in editing mode signalled by the gray background and the presence of reaction and metabolite control points (black and dark blue dots). The illustrated Citric Acid Cycle subsystem is from the Chinese Hamster Ovary (CHO) cell.

reaction edges associated with a reaction to be coincident with the corresponding reaction edge.

- **Metabolic Translation:** A metabolite can be translated by clicking and dragging a metabolite (yellow) circle (Figure 3.3), which changes to a red circle to indicate dragging is active. It restores the default yellow color as soon as dragging becomes inactive. Using this tool, metabolites can be arranged around each reaction based on whether they are a substrate or a product, further organizing the network. As shown in Figure 3.4 (1), metabolites nad, pi, h, and nadh are to the left of reaction GAPD, are then translated to what has been shown in the right section of Figure 3.4 (2) for improved readability. It is important to note

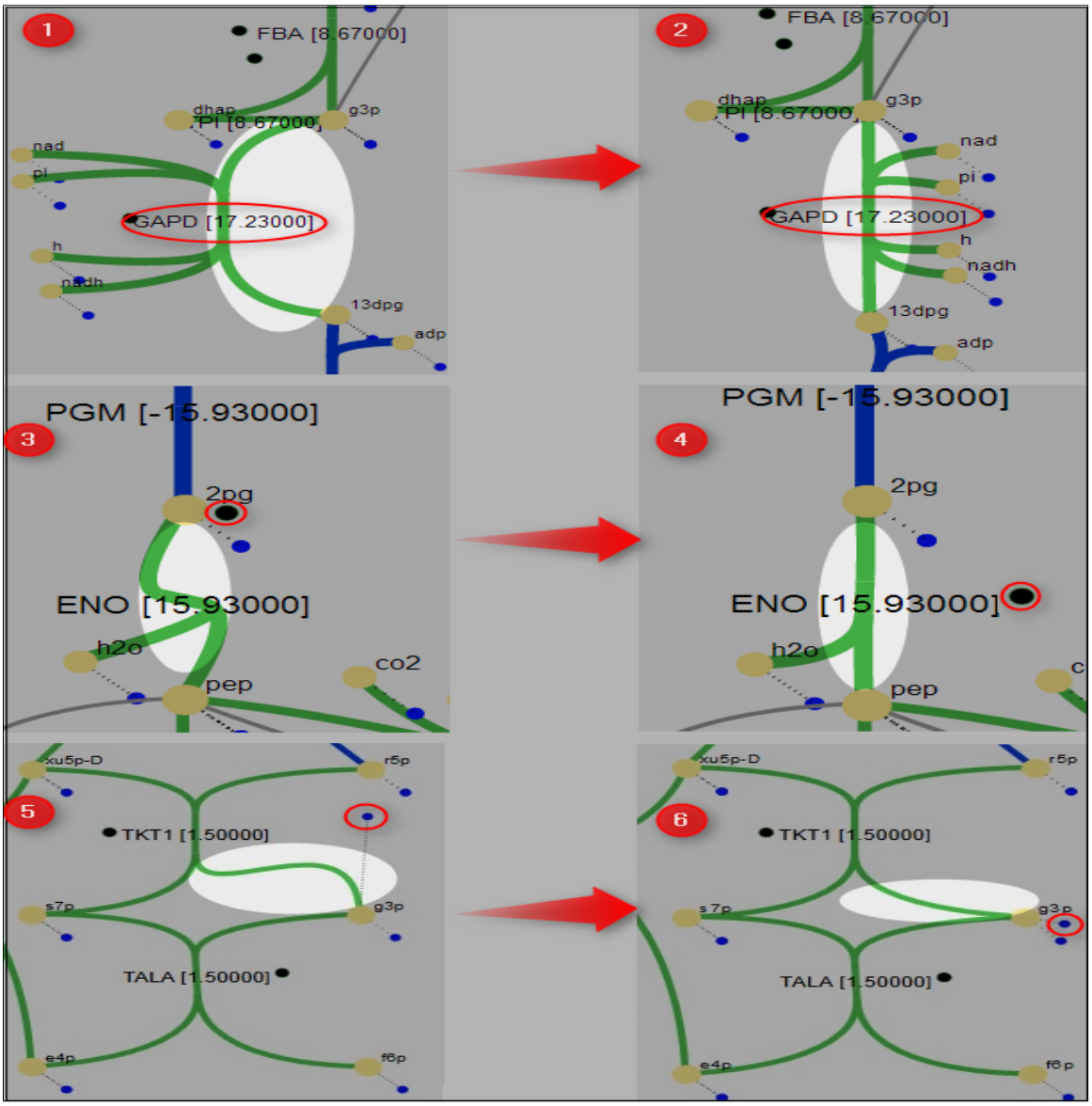

Figure 3.4: Maneuvering operations performed on a reaction using simple drag and drop technique. Left side of the image shows the before operation and right side shows the after operation image. (1-2) Reaction line is translated (3-4) Reaction line is rotated (5-6) Metabolite edge curvature is smoothened.

that while translating the metabolite, the edge curvature control node(blue circle, described later) remains stationary. Once the layout is saved and reloaded, the position of the edge curvature control node is recalculated.

- **Reaction Translation:** A reaction can be translated using its reaction-label. When the translation is active the reaction label turns red and once the destina-

tion is reached and mouse is released the reaction label is restored to its default color black indicating that dragging is inactive. This tool can be used to drag widely spaced reactions (Figure 3.4 (1)) closer to each other as in Figure 3.4 (2). Imposing close proximity aids in visualizing the flux through the reactions. Also, if multiple subsystems are shown on the same screen, this tool is useful for moving all reactions in the same subsystem together. Being able to view all reactions in a subsystem at once is beneficial to understanding the flow of metabolites through the subsystem.

- **Reaction Edge Rotation:** This can be achieved using the rotation handle node i.e. black circle. The angle of rotation can lie anywhere from 0 to 360 degrees. The rotation handle change to red indicating the active rotation and reverts to black when the rotation has been performed see Figure 3.4 (3) and Figure 3.4 (4) reaction edge rotation enables the user to construct cyclic interacting reactions and horizontal and vertical oriented pathways.
- **Edge Curvature Control Node:** A metabolite curvature can be smoothened or translated using edge curvature control points i.e. the blue circle. Brown color indicates that dragging is active and gets restored to blue color once dragging is performed as shown in Figure 3.4 (5) and Figure 3.4 (6). The edge curvature control nodes are useful in adjusting the shapes of reactions. For example, these tools can make a cycle of reactions appear more circular. These nodes are unique to the layout tool and don't appear on visualizations during analysis.

Connectivity Tools

- **Node Cloning:** The term cloning is coined by the tool Arcadia [24] that describes the decomposition of nodes (representing metabolites) with high degree of connectivity into multiple nodes with less degree for the goal to eliminate crossing edges. In PP, a node can be cloned by double clicking on a node, which causes a pop window to appear, where the user selects split node radio button

(Figure 3.5 (1)). The pop up window displays all the reactions associated with this metabolite. On clicking the selected reaction, the metabolite node for that reaction will be cloned (Figure 3.5 (2)).

- **Node Merging:** PP also allows the inverse to the clone operation by providing a metabolite node merge functionality to combine distinct nodes representing the same metabolite into one graph node. In PP, a particular node is double clicked and pops up the merge or split window, merge node radio button is selected (Figure 3.6 (1)), displaying all the reactions associated with the node in a drop down menu from where the target reaction is selected. On clicking the selected reaction, the metabolite nodes for that reaction will be merged see Figure 3.6 (2). Combined these operations allow the user to decompose or merge subnetworks into functional modules through out the course of model development.

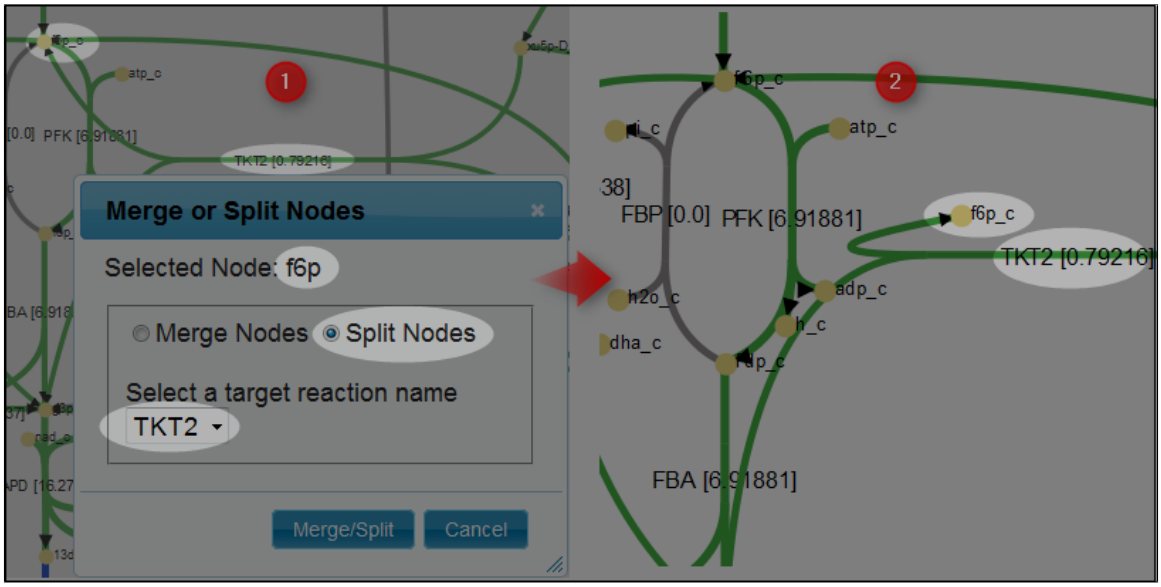

Figure 3.5: In Core subsystem of Ecoli 1366 cell: (1) clone the fructose-6-phosphate (f6p_c) node for transketolase reaction TKT2 and (2) fructose-6-phosphate (f6p_c) after node cloning in TKT2.

Navigation Tools: PP provides with a dynamic and extensive navigation control panel in which user can navigate from a specific subsystem to specific reaction and vice versa.

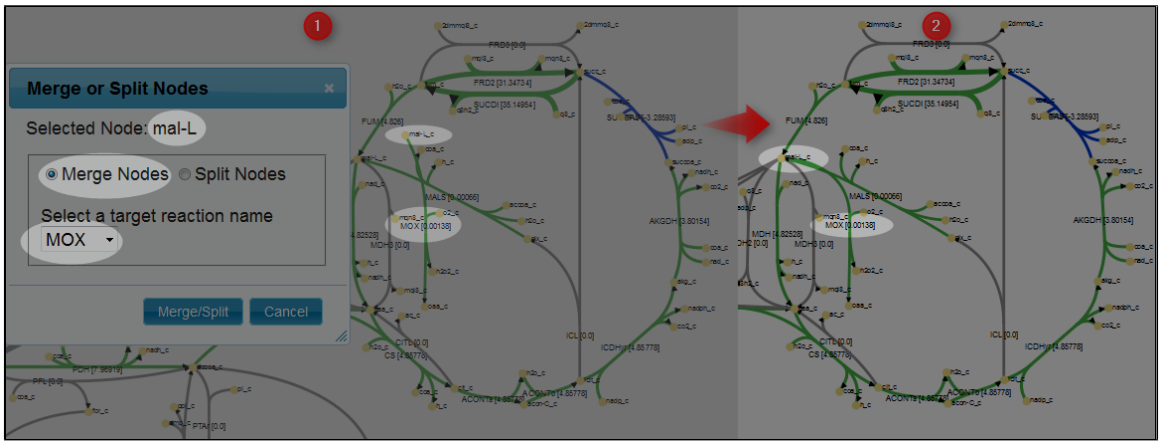

Figure 3.6: In Citric Acid Cycle subsystem of Ecoli 1366 cell: (1) the malate oxidase (MOX) reaction was not connected to any other reaction. The reaction belonged to the citric acid cycle subsystem and included malate (mal-L_c) as a substrate. Three other reactions in the citric acid cycle also shared malate as a substrate, so the malate nodes were merged. (2) The merging of the malate nodes helps complete the citric acid cycle by providing another pathway by which malate can be converted to oxaloacetate (oaa_c)

PP also facilitates the user with zoom and pan capabilities to dynamically select the level of detail. The user can zoom out to any subsystem to lay it out and then zoom in to a specific reaction, or out to see the whole network.

Figure 3.7 illustrates the capabilities of the graph maneuvering tool On the left shows the Citric Acid Cycle of the Cho cell before editing and the right section shows the same network after performing a sequence of translations, rotations, node merge and splits. The result of editing is to create a traditional and understandable Citric Acid Cycle layout.

3.2 Network Import and Export Compatibility

Pathway Pioneer provides a strong network import and export support. It allows the import of metabolic network model encoded in SBML [20] and an easy to use XLS (Excel Sheet) format.

Pathway Pioneer also allows the user to export their modified and customized network in formats JPEG, PNG, TIFF and BMP. PP provides the capability of partial and complete export of a network. A user can navigate to a particular subsystem and then export that partial subsystem only.

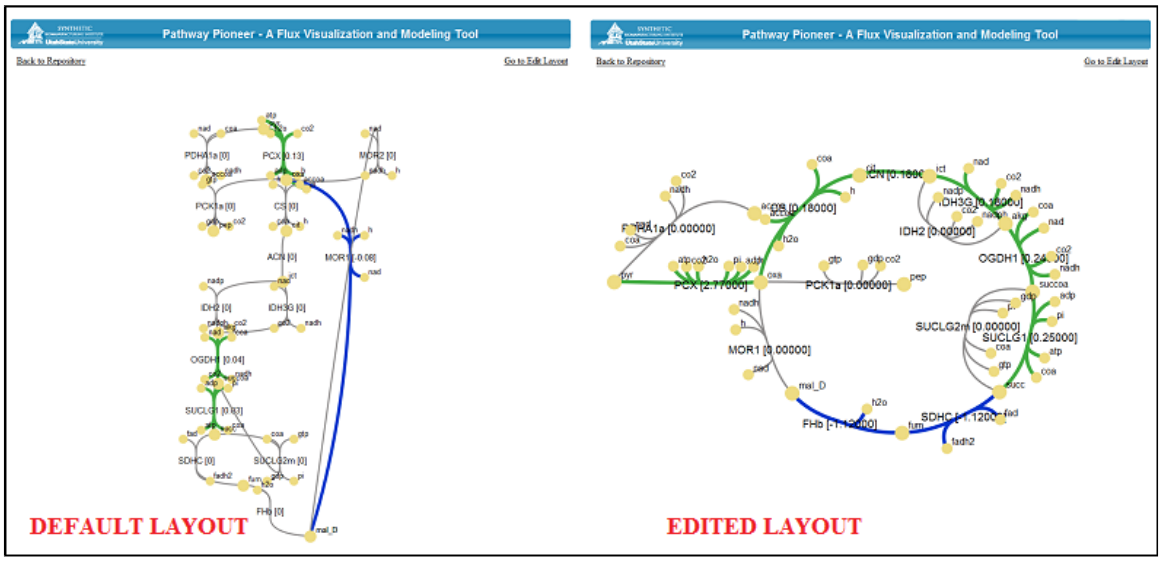

Figure 3.7: Citric Acid Cycle of the Cho cell before and after applying the edit layout feature of Pathway Pioneer.

Pathway Pioneer also provides a platform to publish and share the customized layouts of metabolic networks created by users. A user can publish map files that are used to encode the network coordinates, so they can later be used by any PP user while they upload their models on PP. In addition, PP supports partial layout by which a model can be laid out using the layout map file of a different but related species. Here common reactions are laid out using the map file while new reactions are placed in a regular pattern to enable user editing.

CHAPTER 4

COMPARISON WITH OTHER VISUALIZATION TOOLS

We studied and compared PP with extant state-of-the-art network visualization tools for visualizing and customizing metabolic networks like Paint4Net [23], Patika [25], Medusa [26], Arcadia [24], Cytoscape [15], and iPath2 [27].

Most of these tools are stand alone applications. Medusa [26] is a highly interactive java application for visualization and clustering analysis of 2D biological networks. It provides the user with network editing and exporting features but lacks consistent layout once the network is modified. It also does not support network analysis tools such as Flux Balance Analysis (FBA) [28]. Patika is a multi-user, web-based environment for visualizing and manipulating networks of cellular events featuring force-directed and compounded pathway layout algorithms, but it lacks network customization and lacks an export feature of network in image formats, as well as producing "hair ball" layouts anomaly when it comes to large-scale network. iPath 2 is a tool for visualization, analysis, and customization of various pathway maps. Its interface allows users to navigate and explore the complex pathway maps by zooming and panning controls and also provides visualization of species-specific, manually created maps but on the other hand users cannot modify the layout of the network to customize it according to its choice. To summarize the analysis of all the tools studied, most of the tools are stand-alone systems, support either metabolic network visualization or its analysis, lack encoding of layout map files with inconsistent layout on modifying the network. PP provides users with a set of tools to achieve a high level of subjectivity in laying out of metabolic networks. Combined with PP's analysis tool, it comes up as a complete suite for metabolic network visualization and analysis.

CHAPTER 5

RESULT

Chinese Hamster Ovary (CHO) cells are the most widely used mammalian cell line for industrial production of recombinant therapeutics, with a total global market approaching 100 billion dollars per year. We have shown the application of Pathway Pioneer to layout the *de-novo* synthesized CHO model. After modifying the model (by adding or deleting reactions) the same layout map file can be used to achieve consistent layout of the model during the reconstruction process. Only newly added reactions are automatically laid out, the rest of the reactions are laid out using the existing layout file. In this way PP supports the incremental layout of the models as they are constructed, along with providing the strong feature of exporting and reusing the existing layout map files.

To evaluate the system, a user customized the layout according to the standard conventions employed by researchers for similar systems. We measured the time needed by the user to produce a customized and highly comprehensible layout of the CHO model starting from a layout generated by the automated layout algorithm. Figure 5.1 clearly shows that the time required to create the layout is reasonable, especially considering the significant increase in the utility. When the user incrementally develops a model or if a model is derived or extended from some other parent model for which map file already exist, then only new reactions have to be customized which are not present in the map file of parent model. This feature of pathway pioneer saves user time for network customization. In addition, spent in layout time by one user is a investment from that researcher to the community since this layout map file can be shared and then applied by anyone to layout the same or similar network models.

Figure 5.2 and Figure 5.3 show the customization of the layout of the CHO model by a single user starting from initial layout in Figure 5.2 to finishing with the fully laid out core

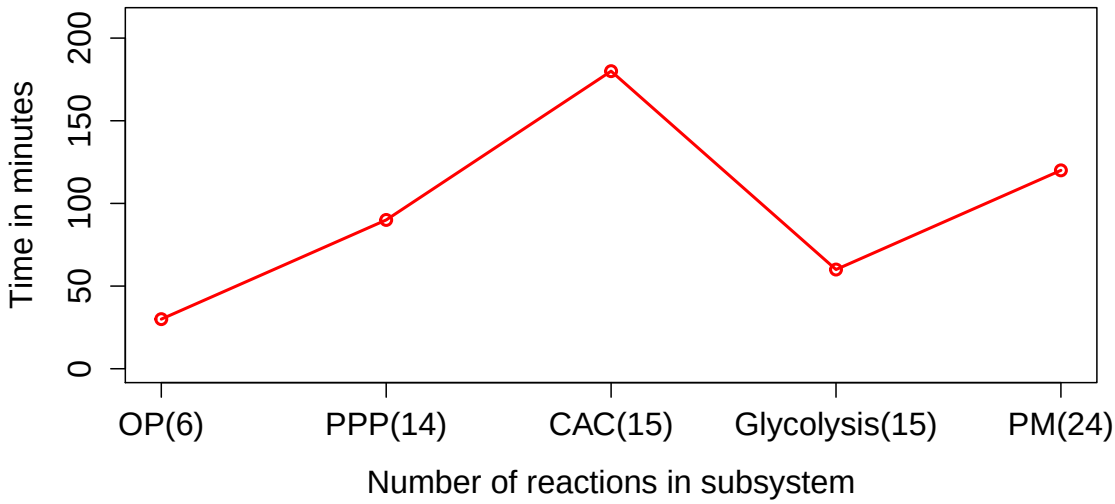

Figure 5.1: Number of reactions laid out vs. time taken for the Cho cell Core subsystem that includes the following subsystems Oxidative Phosphorylation, Pentose Phosphate Pathway, Citric Acid Cycle, Glycolysis/Gluconeogenesis, and Pyruvate Metabolism . This time graph is calculated for a user who customized each subsystem starting from a layout produced by the initial layout algorithm.

subsystem of the CHO model in Figure 5.3.

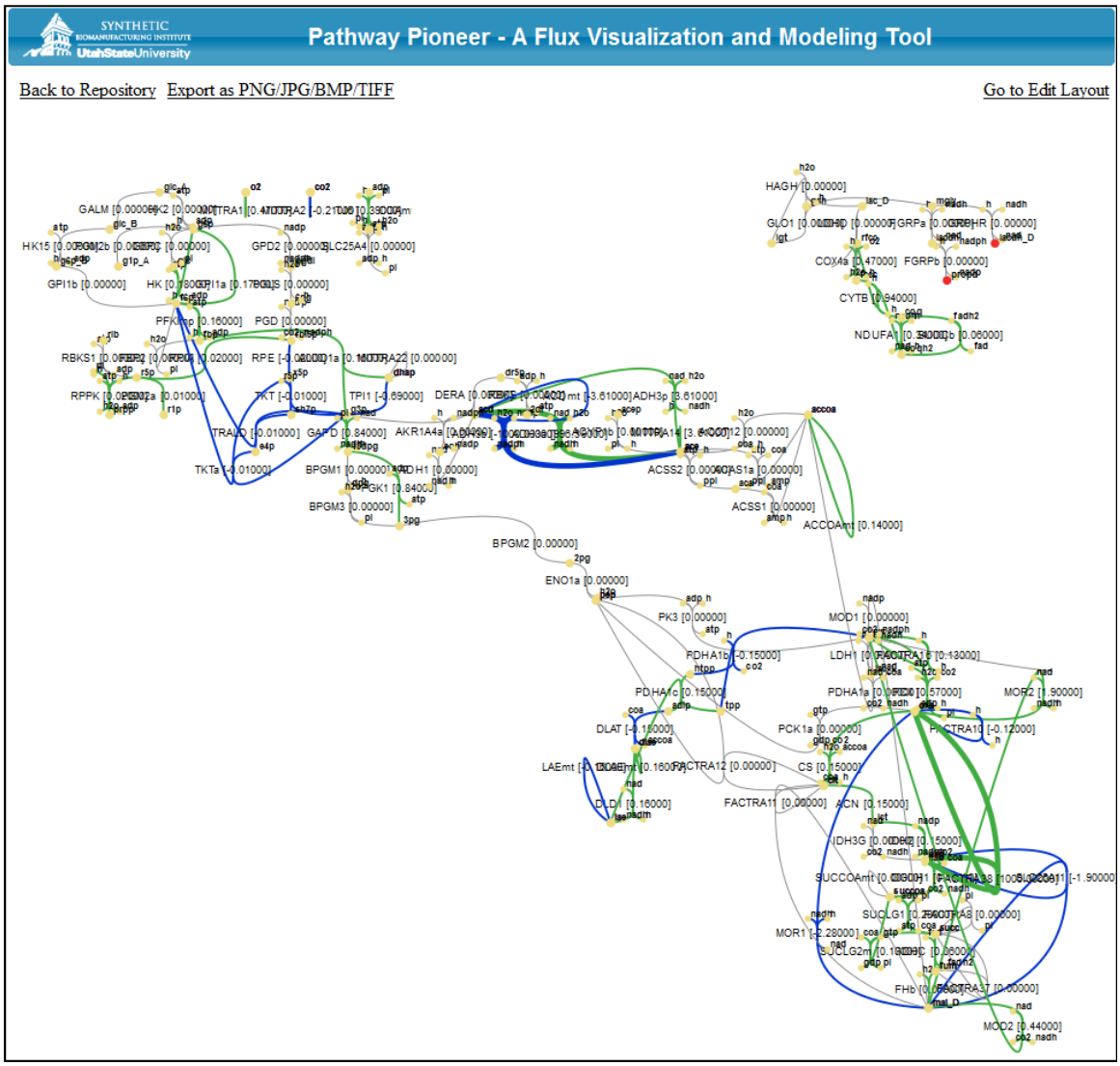

Figure 5.2: The layout of Chinese Hamster Ovary (CHO) Core cell produced using the default layout algorithm. It is evident from this image that this visualization is cumbersome for analysis and study.

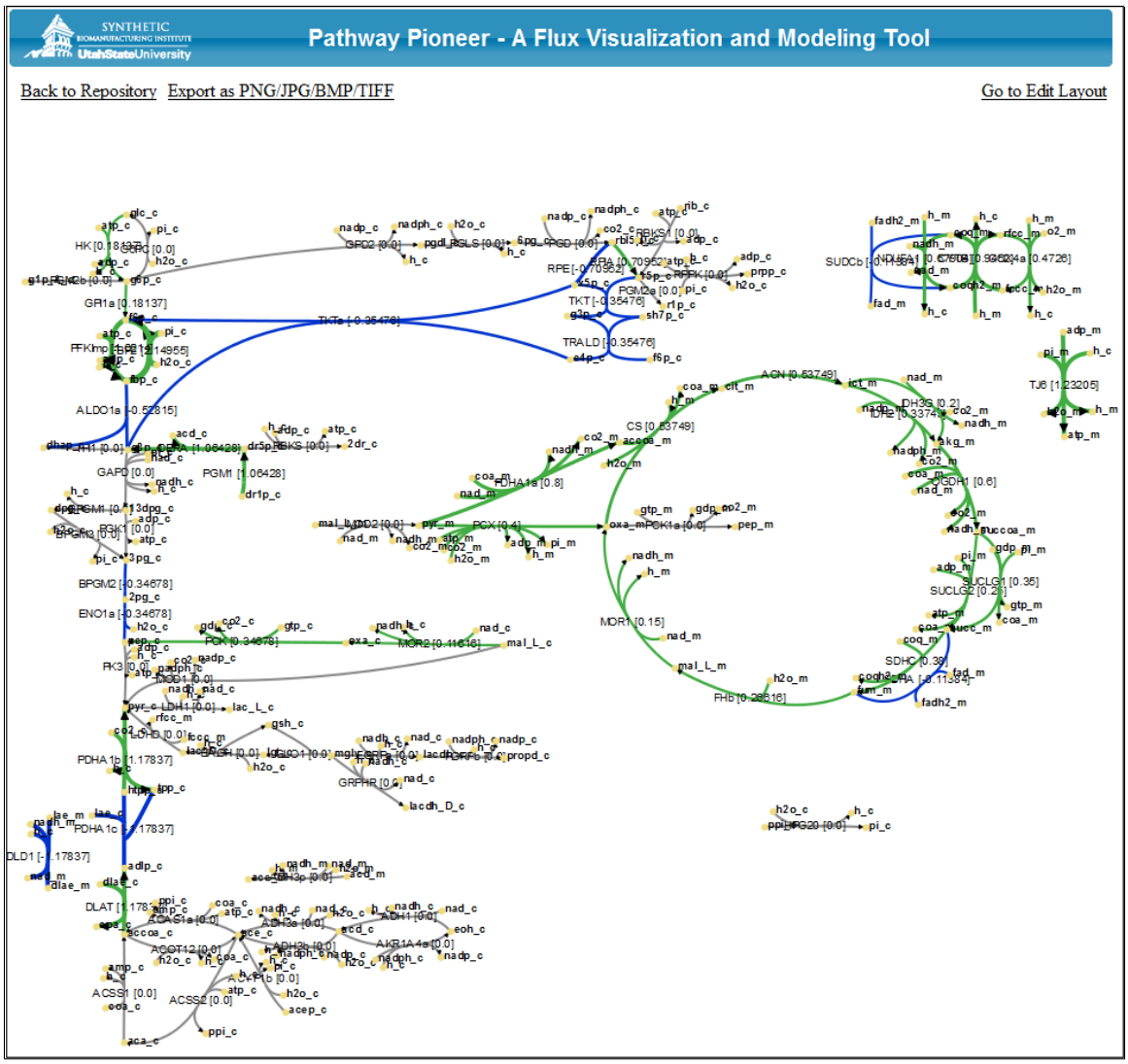

Figure 5.3: The laid out view of Chinese Hamster Ovary (CHO) Core cell after using the interactive graph layout toolbox. The layout map file of this model can be exported and then be used by other users to layout their respective models.

CHAPTER 6

CONCLUSIONS

Extant visualization tools solve a wide spectrum of layout and analysis problems but suffer from the limitations of being stand-alone systems that lack compatibility with other tools and problems of degraded performance and scalability when faced with large networks greater than 1000 reactions. In addition, none combine analysis capability such as FBA with dynamic visualization and layout. Currently no automated method for large scale network layout exists that is optimized for comprehensibility and can be integrated into multiple tools. In fact, an objective measure of comprehensibility does not exist since users have their individual preferences and apply the layout to seek answers to distinct questions.

Pathway Pioneer is designed to solve most of these problems by being a web based tool with cloud storage; it is compatible with the widely used SBML model standards and utilizes the most advanced vector graphics standard SVG for visualization of large networks. Network maneuvering tools enable the user to fully customize the initial semi-automatic layout algorithm to solve the subjectivity problem of large scale network layout according to user choice. PP enables the user with an value added feature of sharing the layout map files with the community, which will help in accelerating research and reducing redundant efforts by different users each laying out similar models.

All these capabilities are designed as a front-end of the widely accepted flux analysis tool COBRA-py, making it a complete biological network reconstruction and layout tool.

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