3D VISUALIZATION AND ANIMATION OF METABOLIC NETWORKS

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ABSTRACT

Metabolic networks represent an important research area in the systems biology field. Simulation is one of approaches used to understand their behaviour, and visualization and animation techniques help the human user to better understand the results of a simulation. In this paper, a novel approach for visualizing metabolic network simulation results is presented. In contrast to previous proposals, this approach makes it possible to animate the timely evolution of a metabolic network in three dimensions, allowing in this way a better interpretation of the results. The relevant issues regarding the proposed animation concepts and their implementation in Java are discussed.

INTRODUCTION

Metabolic engineering is the targeted modification of metabolic pathways by means of genetic manipulations in order to achieve specific goals (Bailey 1991, Stephanopulos and Sinskey 1993). Simulation plays an important role in metabolic engineering. Different models of metabolic pathways are built to describe the metabolism of a cell. Simulators operating on these models generate results which are then compared to *in vitro* experimental data and to *in vivo* data if they exist, in order to select the model which describes these data best. However, the selection process does not only require reliable simulation results but also their correct interpretation.

Visualization is very helpful in evaluating such simulation results. Current visualization tools typically offer 2-dimensional plots which represent the trajectories of concentrations of metabolites and reaction flows and which can be interpreted easier than raw numbers. Nevertheless, it is difficult for the human user to evaluate the relatively large quantities of data for the different phenomena at the same time.

In this paper, we present a new approach which allows to dynamically animate the evolution of a metabolic network based on generated simulation data in three dimensions (3D). This work is a continuation of (Qeli et al. 2003), extending the past approach with a new visualization method to animate the progression of metabolites and reactions in the three-dimensional space. The 3D view allows to avoid some of the difficulties encountered in 2D visualizations. Even the central metabolic pathways, as for example the pentose phosphate pathway, cannot be drawn in 2D without line intersections. A much more difficult problem occurs when the metabolic cofactors like ATP or NADH are involved. They are coupled to almost all central metabolic reaction steps thus inducing a strong network connection resulting in many line crossings. Finally, only a few tools visualize the metabolic regulation (inhibition and activation of reactions by other metabolites). If this is done for a large network, it becomes extremely difficult to keep an overview over the heavily entangled 2D network graph.

This paper is organized as follows. The next section gives a survey of the related work in this field. Then, our new approach to 3D visualization and animation of metabolic networks is presented. Experimental results will be shown in the following section. Finally, some conclusions are drawn and possible future work is discussed.

RELATED WORK

The related work about metabolic network modelling is mainly concerned with different modelling and simulation techniques to simulate the behaviour of a cell. Several commercial or academic tools and frameworks exist which allow to model and simulate metabolic networks. Typically, they consist of the following three components:

1. Design component
2. Simulation component
3. Visualization component

However, in many cases the first and the third component are omitted. This, of course, does not affect the quality of simulation, but the presence of these components makes the evaluation of simulation results much easier.

Gepasi (Mendes 1993, Mendes 1997) is one of the early simulation tools in metabolic engineering. However,

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from the visualization point of view, it offers only common visualization methods in form of snapshots.

SBW (Hucka et al 2001a) is a framework intended to be extensible for running on different platforms to aid people in research in the systems biology field. SBML (Hucka et al. 2001b) is one of their major achievements, representing a standardized XML based format for modelling biochemical or metabolic networks, respectively. Several tools are included in this framework, *Jdesigner* being one of them. This tool allows the interactive design of metabolic networks.

Another tool called *Virtual Cell* (Loew and Schaff 2001) offers similar functionalities like SBW.

INSILICO Discovery (Mauch et al. 2001) is a commercial tool which offers a full fledged simulator and also allows the user friendly design of metabolic networks. The design is a mixture of manual interaction with different graph drawing techniques adopted for metabolic networks. Some of these techniques are described more precisely in (Karp and Paley 1994) and (Becker and Rojas 2001).

FluxAnalyzer (Klamt et al. 2003) is a MATLAB package which besides offering several functionalities for analyzing the structure of metabolic networks allows the visualization of so called interactive flux maps, which are a first step towards interactive visualization of metabolic networks.

MetVis (Qeli et al. 2003) offers a new approach for visualizing metabolic networks. It allows not only their interactive design, but also their animation in two dimensions according to results generated via simulation. The simulator used is MMT (Hurlebaus 2002; Haunschild 2002) which generates simulation code based on pulse experiments.

Dwyer et al. (Dwyer et al. 2004) present a similar approach where experimental data is visualized directly in a metabolic network. This work is a continuation of (Brandes et al. 2003) where related metabolic networks are superimposed over each other to create a 2 ½ dimensional view of metabolic networks.

MNV (Rojdestvenski and Cottam 2002; Rojdestvenski 2003) visualizes metabolic networks by means of VRML (Virtual Reality Markup Language) to allow a 3D view. However, it is somewhat difficult for a biologist to get accommodated with the visualization as the transformation from 2D to 3D is difficult to comprehend from a biological point of view.

OUR APPROACH

The approach we present in this paper is realized as part of *MetVis* (Qeli et al. 2003), but can also function as a separate application. Furthermore, *MetVis* is part of a framework which includes a simulator called MMT written in C++ (Hurlebaus 2002; Haunschild et al. 2002). The exchange between *MetVis* and MMT is achieved by using XML documents for metabolic models and CSV (Character Separated Value) for

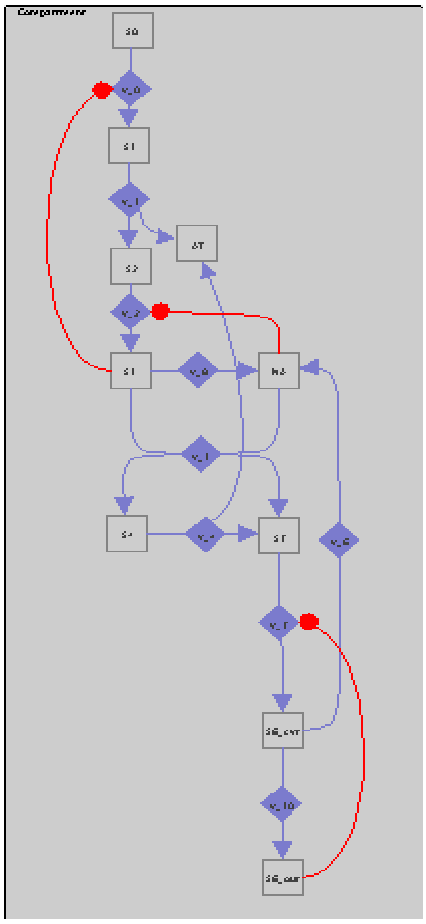
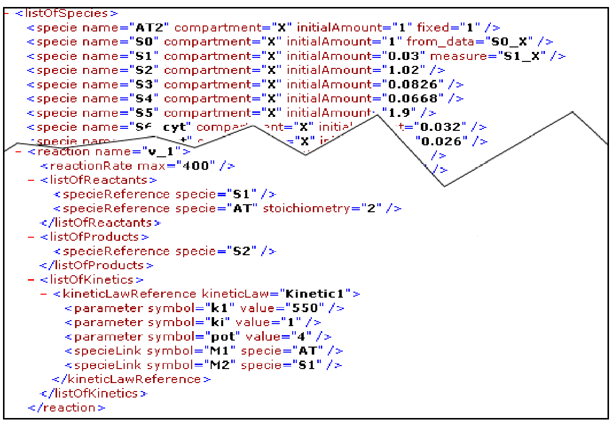


Figure 1: A Fictitious Metabolic Network

simulation data. From a graph theoretic point of view, a metabolic network is stored as a directed bipartite graph with two types of nodes: metabolites and reactions. The edges of the graph show which metabolites take part in a certain reaction. Another approach could be to model the metabolic network as a hypergraph.

Figure 1 shows a fictitious metabolic network designed in 2D, and figure 2 shows its corresponding XML fragment. Metabolites are represented by squares and reactions by rhombuses. The flows are represented by Bezier curves.



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- <listOfSpecies>
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    <specie name="S0" compartment="X" initialAmount="1" from_data="S0_X" />
    <specie name="S1" compartment="X" initialAmount="0.03" measure="S1_X" />
    <specie name="S2" compartment="X" initialAmount="1.02" />
    <specie name="S3" compartment="X" initialAmount="0.0826" />
    <specie name="S4" compartment="X" initialAmount="0.0668" />
    <specie name="S5" compartment="X" initialAmount "1.9" />
    <specie name="S6 cyt" compar  ent="X" initia   t="0.032" />
    <specie nam      t" c     ="X" ir        "0.026" />
  - <reaction name="v_1">
      <reactionRate max="400" />
    - <listOfReactants>
        <specieReference specie="S1" />
        <specieReference specie="AT" stoichiometry="2" />
      </listOfReactants>
    - <listOfProducts>
        <specieReference specie="S2" />
      </listOfProducts>
    - <listOfKinetics>
      - <kineticLawReference kineticLaw="Kinetic1">
          <parameter symbol="k1" value="550" />
          <parameter symbol="ki" value="1" />
          <parameter symbol="pot" value="4" />
          <specieLink symbol="M1" specie="AT" />
          <specieLink symbol="M2" specie="S1" />
        </kineticLawReference>
      </listOfKinetics>
    </reaction>
```

Figure 2: XML Model of the Network of Figure 1

The XML format we use is a dialect of SBML (Hucka et al. 2001) which also supports model variants (Haunschild et al. 2002). The graphical information related to the metabolic network is stored in another XML file. This allows to associate several graphical representations with the same metabolic network (Qeli et al. 2003).
The simulation data is obtained in form of a CSV file from MMT. In contrast to (Dwyer et al. 2004) where experimental data (the analogue of our simulation data) is stored directly in the graph data file, we have it separated to allow viewing the results of several simulations of the same metabolic network.

3D Visualization

2D visualization of metabolic networks represents a big step forward in the analysis of their functionality. Currently, it is the favourite approach in designing metabolic networks from the biological point of view. However, it has several drawbacks, one of them being the complex view that is created for large networks due to crossings that are created in two dimensional views. One way of eliminating crossings is to duplicate metabolite nodes, but there are no clear rules how often duplication should be done. Additional edges like the ones representing activation or inhibition relationships between a metabolite and a reaction complicate the situation further.
We have developed a new approach as part of MetVis which creates a 3D visualization based on a 2D visualization. The 3D view is intended to be used as a complementary part to the 2D view. The third dimension not only allows the elimination of crossings in 2D, but also allows us to visualize several metabolic networks in the same view for comparison purposes. Furthermore, the generated 3D views are similar to their 2D counterparts, without creating a totally different view which would confuse modelers in their work.
In the proposed approach, metabolites are represented by 3D cubes, and edges that in 2D were represented by Bezier curves with 4 control points are now represented by tubes that have the shape of a three dimensional Bezier curve and with a certain diameter. To eliminate crossings between edges, the two middle control points are displaced in different z-planes. Figure 3 presents the 3D visualization of the example shown in Figure 1, where the edges of the reaction S1+Na-> S4+S5 are in plane z=0 and those of S4 -> S5+AT are displaced with a constant c. A separate z-plane is used for inhibitor and activator edges such that they do not visually affect the rest of the network, as shown in Figure 3.

3D Animation

To animate the 3D views, the raw data taken from a simulation is converted into relative percentages of the respective metabolite or flow, such that the values are in a certain range and are comparable to each other to

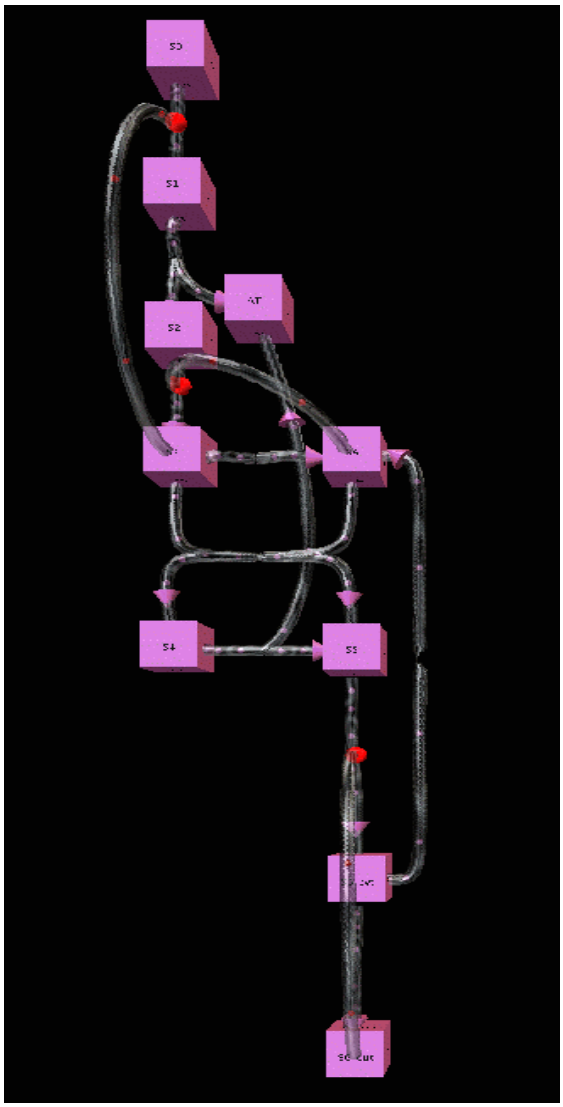

Figure 3: 3D Visualisation of the Model of Figure 1

avoid drawing false conclusions from their visualization. For each metabolite or flow X at time t, the percentage value of the respective metabolite/flow is calculated as follows:

$$Percentage(X,t) = \frac{Value(X,t) - Min(X)}{Max(X) - Min(X)} \quad (1)$$

where Value(X,t) represents the value of metabolite X at time t and Max(X) and Min(X) represent the minimum and maximum value for each metabolite/flow during the entire simulation. Two types of objects are animated:

- For the cubes representing the metabolites, their z dimension varies according to the concentration of the corresponding metabolite calculated according to formula (1).
- Tubes represent flows for both reactions and inhibitions, and accordingly colored spheres moving within the tubes represent the material flow for the corresponding reaction. For the reaction flows, these spheres are colored with a nuance of blue, for inhibition

they are colored in red and for activation they are colored in green. The speed of flow of these spheres depends on the percentage speed of the respective reaction calculated according to formula (1).
Depending on the point of time, cubes will show how high the concentration of the metabolite is, and the reaction flow will be faster or slower.

Implementation

The approach has been implemented completely in the Java programming language. For 3D rendering, the idx3d library (Walser 2000) is used. This library is similar to the well know Java3D library, but offers additional functionality for our specific case and is in pure Java allowing easy inclusion in Java applications and applets without additional libraries.
The application consists of 20 Java classes. The I/O package is reused from MetVis. To allow effective user interaction and to achieve better performance, threads are used, which allow parallel processing of the rendering process. These threads are organized in a pool of threads, in order to keep the overhead of the administering thread low.
It is worth mentioning that for eliminating crossings, a simple but effective algorithm was implemented to assign different z-planes to the middle control points of bezier tubes. The nodes of the metabolic network (i.e. reactions and metabolites) are all left in plane z=0. Only the two inner control points of Bezier curves (i.e. edges) that connect metabolites with reactions are shifted to different z-planes. The edges are processed consecutively. In the beginning, the first edge is directly assigned to the first group of edges to be drawn in plane z=0. Then, the second edge is processed; if it intersects with the first edge, a new group is created with edges to be drawn in plane z=c, otherwise it is inserted in the first group. The third edge is processed in the same way, if it intersects any of the edges in the first group or second group (if this group exists), then a new group is created with edges to be drawn in plane z=-c, otherwise it is inserted in the first group where it does not intersect with other edges. The fourth edge would be drawn in the plane z=2c if it intersects with at least one edge from every previous group, the fifth in plane z=-2c, and the algorithm proceeds in this way until all edges are processed.

EXPERIMENTAL RESULTS

Our project partners of the Institute of Biotechnology in the Research Center Jülich, Germany are the ones who create new models, simulate them, evaluate the simulation results and do experiments to see the correctness of their hypotheses. In figures 4 and 5 we see one of the models of a part of the metabolism of E.coli built in Jülich. Figure 4 shows a randomly selected step of the animation in 2D, and Figure 5 shows the same step in 3D. The advantages of 3D are

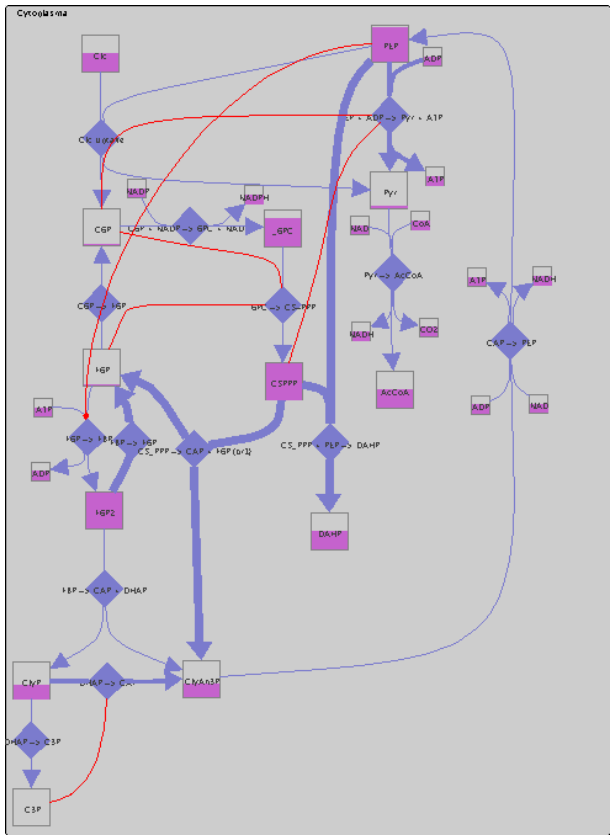

Figure 4: 23rd Step of a 2D Animation of a Partial Model of E.coli

not directly clear from the screenshots; a demo version may be obtained from the authors to view them in action. For example, whereas in the 2D animation one must be concentrated to view the changes in different flows, in 3D animation the speed of the movement of spheres makes it directly clear which part of the metabolic network is more active.

CONCLUSIONS

In this paper, we have presented a new approach to animate metabolic networks in three dimensions. In contrast to previous approaches, this approach allows the dynamic visualization of the evolution of a metabolic network model based on data generated via simulation. The approach could also be used to animate other kinds of networks where information about the flows in the network is available e.g. the visualization of regulatory networks in general.
There are several areas for future research. Currently, we are experimenting with different layout algorithms in two dimensions. The next step would be the adoption of these algorithms for the 3D space to increase the clarity of the visualization of metabolic networks but also remain consistent with the 2D representations biologists are used to. Highlighting specific pathways of a metabolic network and animating selected parts of a certain metabolic network is also a direction we are

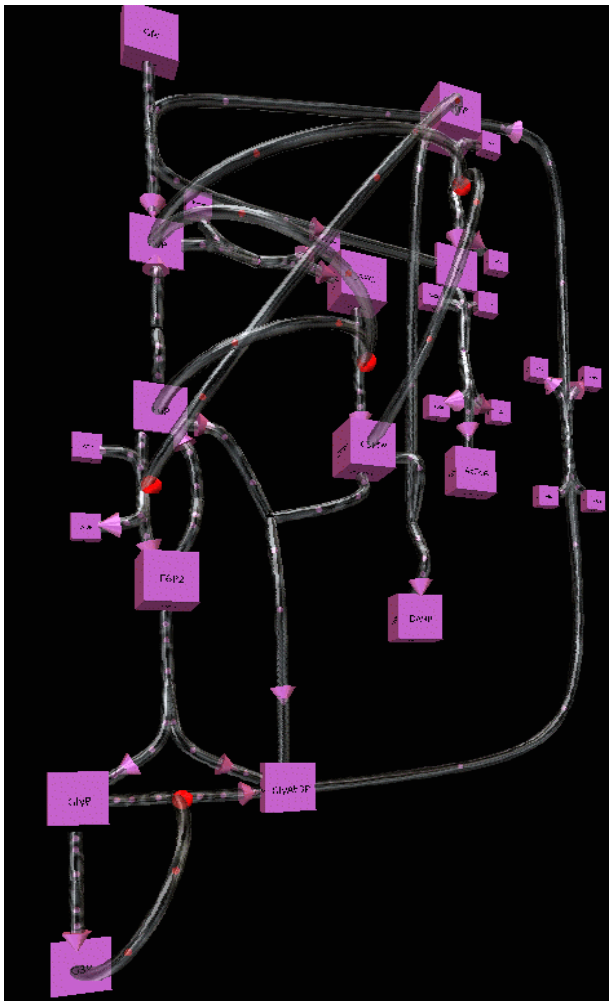

Figure 5: 23rd Step of 3D Animation of a Partial Model of E.coli

currently working on. Finally, it would be interesting to present several 3D animations on the same screen to create in this way a "3 ½ dimensional visualization" for comparing different animation results.

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