CELLMICROCOSMOS 4.1
An Interactive Approach to Integrating Spatially Localized Metabolic Networks into a Virtual 3D Cell Environment

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Abstract: The high potential of Bioinformatics research concerning quantitative and qualitative data acquisition such as data warehouses, spatial structure prediction and 3D microscopy conveys the vision of generating a computational virtual cell. This paper discusses an approach which allows the creation and exploration of an abstract compartmented cell environment, which can be used for (semi-)automatic, species- and organelle-specific mapping and the comparison of metabolic data.

1 INTRODUCTION

During the last few years initial alternatives to the well-established 2D view of networks have been developed. The growing amount of data led to the idea of introducing techniques to extend the two visual dimensions widely used in the scientific community by a third one: Biological pathways are now visualized with different layout algorithms to give a better understanding of the biological data structure. On the other hand there is the cell visualization and simulation area which already partially deals with three dimensional cell models, but none of these approaches tries to visualize compartmental interrelationships cell internally in 3D.

CELLmicrocosmos 4.1 PathwayIntegration (CmPI) introduces a novel approach of interactively combining abstract or 3D Microscopy based cell components with metabolic networks. To take full advantage of 3D visualization, different comparing methods and layouts, a user-optimized navigation and 3D Stereoscopy have been integrated. To support the correlation of organelle internal localization levels with the involved enzymes, information provided in the databases *BRENDA* (Chang et al., 2009) and *UniProt* (The UniProt Consortium, 2008) are obtained.

The *CELLmicrocosmos (Cm)* project is intended to develop tools to provide the creation and exploration of synthetic cell environments for different purposes. It is intended to be applied to

scientific as well as educational purposes in the future.

2 RELATED WORKS

The scope of the application introduced here is defined by two main areas: the pathway visualization in 3D and the cell visualization and simulation.

2.1 Pathway Visualization in 3D

One of the initial approaches to visualize metabolic pathways in 3D was already aimed at the avoidance of unaesthetic aspects known from 2D visualizations like line crossing and the reduction of topological constraints (Rojdestvenski, 2003). Additionally, it provided an opportunity to export the results to *VRML (Virtual Reality Modeling Language)* and followed the *Focus+Context paradigm* (Robinson & Flores, 1997): The user can focus on a point of interest (POI) while still able to keep track of the complex network structure. The mentioned features are also implemented in *CmPI*.

Another approach lies in the *2.5D Visualization* of metabolic networks (Fung et al., 2008), which offers comparison methods for two different biological networks: On the first 2D layer a metabolic pathway is presented, on layer two a protein interaction network and on the third layer, located in the middle of the 3D space, the overlapping nodes are shown. In other 2.5D

visualization approaches the layer concept is used for the inter-organismic (Brandes, Dwyer & Schreiber, 2004) or inter-domain large-scale (Pavlopoulos et al., 2008) comparison of related metabolic networks. *CmPI* distributes pathways of different organisms in three dimensional space and also integrates a layer paradigm, but focused on the spatial segmentation of cellular components. Another analogy with those 2.5D approaches is the use of *KEGG* (Kanehisa et al., 2008) as the metabolic data source.

MetNetVR introduced a possibility of visualizing complex large-scale, hierarchical networks interactively by implementing different 3D layout algorithms (Yang et al., 2006). Virtual Reality techniques are used to extend displays into the third dimension. For *CmPI*, 3D Stereoscopy has been integrated which is compatible with workstation as well as professional back-projection concepts. In addition, the network layouts of *MetNetVR* may follow the cellular compartimentation. But in comparison to the *CmPI* approach, it is not possible to distinguish between different cell component layers or to integrate different cell component models and localizations interactively.

BioCichlid is another tool which visualizes and animates time-dependent gene expression data, correlated with protein interaction, signalling and regulatory networks in 3D (Ishiwata et al., 2009). *CmPI* does not implement time-related data so far.

2.2 Cell Visualization and Simulation

Cell simulation environments have been extended from 2D to 3D during the last few years:

CompuCell3D is a software framework to simulate the development of multicellular organisms with stochastic rules and differential equations (Merks & Glazier, 2005). *E-Cell3D* is implementing meta-algorithms also based on differential equations to simulate nonlinear interactions between functional modules. In addition, parallelization of complex mathematical analysis is provided (Sugimoto et al., 2005). The *Virtual Cell simulation environment (VCell)* allows the formulation and simulation of cell biological models in 3D (Loew & Schaff, 2001).

The cell models of the mentioned approaches are based on a very high grade of simplification, because the effort for the computation increases with the model complexity. In contrast, *CmPI* focuses on a single cell at a structurally higher detail level, taking cell components and their layers into account, but it is not intended as a simulation environment so far.

Meta!Blast is a pedagogic entertainment software in development. The aim is to create a

gaming experience for students, which links the molecular and anatomic level (Call et al., 2006).

3 IMPLEMENTATION

3.1 The 3D Cell Environment

For many visualization applications it is sufficient to integrate abstractly designed cell components which are based on 2D electron and/or confocal laser microscopy scans, supporting a clear data arrangement and high-performance rendering. Because the results of 3D microscopy are improving and partly made freely available to the community (Martone et al., 2002), we exemplarily integrated a mitochondria model based on 3D electron tomography (Figure 2) to create a linkage between abstract/functional and realistic/structural data.

The cell components are imported as *VRML97* models and provide different layers. For instance, the mitochondrion consists of five shapes featuring capability to map data to the matrices, the inner/outer membranes, the intermembrane spaces and clouds (Table 3).

3.2 Data Integration

The system should be open to make use of a wide range of data sources: It connects to a data warehouse integrating a number of life-science-relevant databases: *BioDWH* (Töpel et al., 2008).

Metabolic data and their relations are visualized as networks that are generated by an integrated version of *KEGG* (Kanehisa et al., 2008). The 3D cell environment contains enzymes and compounds (products and substrates) which are involved in different metabolic pathways. The connecting lines show the reactions including the direction.

The localization information needed for the spatial placement of enzymes is retrieved from *BRENDA* (Chang et al., 2009) and *UniProt* (The UniProt Consortium, 2008).

3.3 Data Mapping

3.3.1 Cell Component Related Mapping

Table 1 shows an overview of automatic localization results coming exclusively from *BRENDA* or *UniProt*. Best results are achieved by combining the data for the Citrate Cycle: All enzymes are localized (Table 2).

Focussing exemplary on mitochondria, *UniProt* contains more than 50, *BRENDA* more than 20

different localization definitions; Table 3 shows in the left column an aperture.

Table 1: A comparison between the results achieved exclusively from *BRENDA* or *UnitProt* for homo sapiens.

Pathway	BRENDA	UniProt
Glycolysis	62%	70%
Citrate Cycle	70%	82%
Fatty Acid Biosynthesis	33%	16%
Fatty Acid Elongation in Mitochondria	100%	80%
Fatty Acid Metabolism	91%	71%
Pyruvate Metabolism	70%	70%

Table 2: An overview of combined localization results coming from *BRENDA* and *UnitProt* for homo sapiens.

Pathway (KEGG identifier)	Localization
Glycolysis (hsa00010)	22/24 (91%)
Citrate Cycle (hsa00020)	17/17 (100%)
Fatty Acid Biosynthesis (hsa00061)	2/6 (33%)
Fatty Acid Elongation in Mitochondria (hsa00062)	5/5 (100%)
Fatty Acid Metabolism (hsa00071)	12/12 (100%)
Pyruvate Metabolism (hsa00620)	19/20 (95%)

Investigating definitions, the problem evolves that different terms belong to the same localization. The mapping information for *CmPI* is stored in a HashMap. For example 'mitochondrial inter-membrane space' and 'mitochondrial lumen' are both mapped onto the 3rd mitochondrial layers (Table 3, right column).

Table 3: An exemplary comparison of the mitochondrial localizations found in *BRENDA* and the mapping to cell components in *CmPI*.

BRENDA Localization	CmPI Localization
mitochondrial matrix	1. Matrix
mitochondrial inner membrane	2. Inner Membrane
mitochondrial respiratory chain complex I	
mitochondrial intermembrane space	3. Intermembrane Space
mitochondrial lumen	
mitochondrial outer membrane	4. Outer Membrane
mitochondria	
mitochondrial cloud	5. Cloud

3.3.2 Localizing an Enzyme

An interactive localization table offers opportunities to compare and inspect corresponding references and, if necessary, to adjust the automatic localization settings. Loading the Citrate Cycle for *Homo Sapiens* from *KEGG*, the enzyme 1.2.4.2 *(Oxoglutarate dehydrogenase)* will appear in the table in addition to other enzymes. *CmPI* provides

all organism- and enzyme-specific localizations derived from *BRENDA* and *UniPro* and also allows the user to predict manually the localization.
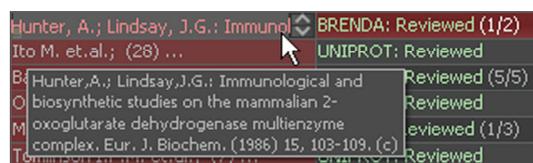

Figure 1: Using *BRENDA* for the localization of EC 1.2.4.2, the term 'mitochondrial inner membrane' was extracted from the publication shown in the Pop-up frame and in the left column. The number in brackets indicates the different publications pointing to the same localization. In the right column the number of different localizations is shown. For the selected enzyme, two localizations have been found and the first one is selected.

The user can choose onto which cell component the enzyme should be placed. There are three qualitative mapping options:

- Reviewed Localization (*BRENDA* and *UnitProt*): The database entry has been reviewed by a curator.
- Unreviewed Localization (*UnitProt*): The database entry has not been reviewed.
- Predicted Localization: No database entry has been found, the user has to localize manually.

In addition the user can decide onto which cell component layer the enzyme should be mapped. If the localization term is imprecise, there are two options for the user to check the localization: He may read the commentaries which are sometimes provided by the database and shown in the localization table or he may review the publications (Figure 1), which are additionally often directly linked to the abstracts of the *PubMed* webpage.

If the database entry contains only 'mitochondria' and there are no additional comments coming from the database or the publication, the user has to predict the layer. In case of the mitochondrion, *CmPI* automatically maps an enzyme with the term 'mitochondria' onto the outer membrane (Table 3).

Even if there is a precise localization term, it may be reasonable to read the corresponding publications found in the localization table: For enzyme 1.2.4.2 Hunter and Lindsay (1986) (Figure 1) mentioned, that it might be localized at the mitochondrial inner membrane as well as the matrix. If there are too few localization results for some organism/pathway combinations, the user can download the localization for all organisms and try to search for comparable results.
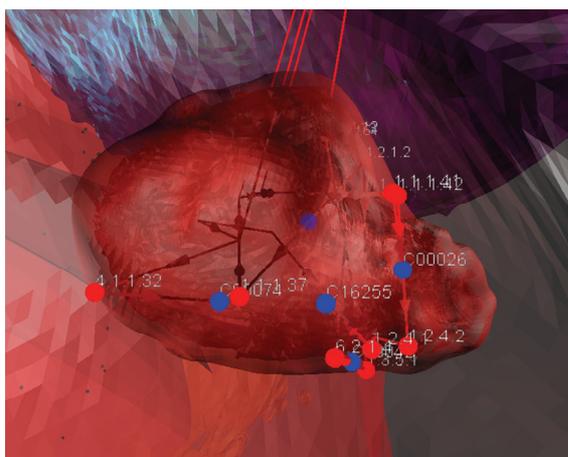
Figure 2: A mitochondria model manually segmented and modelled from a tomographic reconstruction of isolated liver mitochondria from Yamaguchi et al. (2008), correlated with the citrate cycle of homo sapiens. The importance of a well-defined navigation is shown by the spatial complexity: It dissolves when navigating in 3D, especially if 3D Stereoscopy is used. Figure 3 shows the same network in 2D.

3.3.3 Placing Nodes in 3D Space

For the mapping of enzyme nodes in 3D space the *Inverted Self-Organizing Graphs (ISOM)* layout algorithm was adapted, distributing nodes onto Unit Hypersphere (Meyer, B., 1998). This ensures that connected nodes are placed in proximity to each other. Because these nodes need to be mapped onto partially complex-shaped cell components, they are placed first on a surrounding sphere. Then they are moved to the location lying on the shape between the original computed *ISOM* point and the center of the cell component. The compound nodes are placed dependent on the position(s) of the connected enzyme(s). For the placement of cell components as well as the nodes we utilized a seed-based random number generator, ensuring a consistent spatial placement.

3.4 Spatial Data Browsing

3.4.1 3D View and Navigation

An easy-to-use and powerful six degrees of freedom *(6DoF)* navigation offers three different modes:

The *Floating Mode* provides keyboard-based movement in the 3D view and a free mouse cursor for cell component and node interaction, e.g. selecting and jumping to an object. Enabling the *Flight Mode* locks the mouse cursor in a centered position. Vessel-like behaviour allows an intuitive
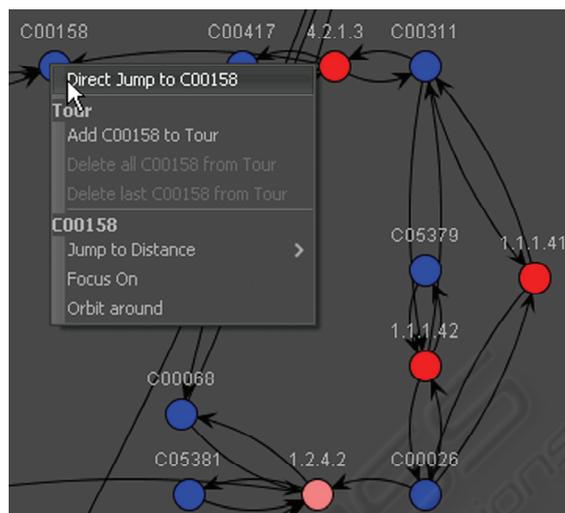

Figure 3: The 2D Visualization of the pathway based on the *KEGG* layout. The Pop-up menu shows options for the clicked node. The active node is EC 1.2.4.2.

navigation through the cell. Mouse buttons accelerate and mouse movement rotates the view. To provide exploration of single cell components and nodes, the *Object-Bound Mode* allows binding the navigation to such an element. The view orbits around the bound object. The mouse cursor is free for interaction and provides movement via click and drag. Another implemented features is a tour editor. To take full advantage of the 3D perspective, 3D Stereoscopy is implemented, compatible to e.g. nVidia® Quadro® FX cards.

3.4.2 2D View and GUI Correlation

Additionally, a 2D view has been created by using the *JUNG* library (O'Madadhain, 2009). In order to give biologists a well-known alternative interface, the *KEGG* map layout can be used to navigate through 3D space (Figure 3).

Following the *Focus+Context paradigm* (Robinson & Flores, 1997), Pop-up menus are integrated in the 2D and 3D view as well as in the localization table. The program provides a well-defined correlation between GUI and the 3D environment. Clicking on a 3D node triggers a panel showing related information, e.g. alternative names, references for the localization, links to open a browser with corresponding websites and to neighbor nodes. These links can also be used to navigate in the 3D view. The localization table also marks the active enzyme and pathway.

3.5 Comparing Methods

To compare the localization of enzymes involved in different pathways, it is possible to load different pathways into one cell environment. The edges of those pathways are drawn in different colors (Figure 4). Type-identical Enzymes at the same cell component have the same position (Section 2.3.3). If the user does not want different pathways to be placed on the same cell component, he may build a cell environment containing multiple copies of a specific cell component which are lying parallel to each other (Figure 5).
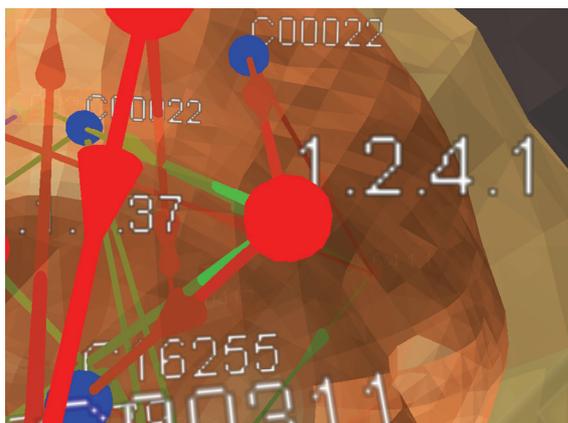

Figure 4: EC 1.2.4.1 (Pyruvate Dehydrogenase) involved in the Citrate Cycle (green) and the Pyruvate Metabolism (red) correlated with a more abstract mitochondria model.

3.6 Data Exchange

Import and export capabilities are provided for cell compositions by packaging a native *XML* format with linked *VRML* cell component models (suffix: .Cm3) as well as for metabolic pathways including all localization settings (suffix: .Cm4). The whole model can be exported in *VRML97* in order to be used by other 3D visualization programs.

3.7 Implementation Details

The webstart compatible project is implemented in Java 6/Java3D. It needs only Java preinstalled and an internet connection for the initial downloading and optional updating process. The *CmPI* module is integrated into the Cm 1 *CellExplorer* application, also includes the Cm 3 *CellEditor* module providing capabilities to create abstract cell environments by using different cell component VRML models.

The Webstart application is located at:
http://Cm4.CELLmicrocosmos.org

4 RESULTS

We combined two substantial areas of Bioinformatics: Cell and Network Visualization. For this purpose we developed a framework which enables researchers to localize, visualize and explore organism- and organelle-specific enzymes in 3D space.

The data integration of *KEGG* ensures a broad application area. The localization of enzymes using *BRENDA* and *UniProt* achieved good results for the organelle specific mapping. Because the database entries are often imprecise, the automatic mapping at the organelle internal layer is still problematic. To dissolve the spatial complexity of networks in 3D, the *ISOM* layout algorithm and a *6DoF* navigation has been implemented. Additionally, we showed the potential of linking abstract/functional and realistic/structural data by correlating pathways with 3D models derived from 3D microscopy.
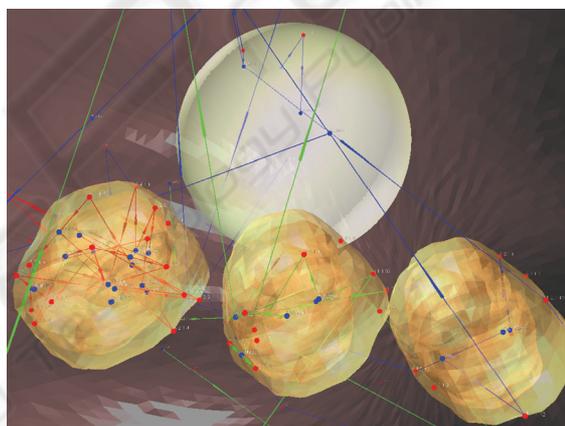

Figure 5: Left mitochondrion: Citrate Cycle, Center: Pyruvate Metabolism, Right: Glycolysis; Some nodes are localized at the cell membrane (red), the cytosol (transparent) and those not localized with the white sphere.

5 OUTLOOK

To improve the qualitative and quantitative amount of localization data, alternative databases should be integrated, providing data of different organisms and reliability or even different types of networks, for example signalling and/or protein-protein interaction data. The network visualization will be improved by adding applied layout algorithms.

Another exciting challenge to take is the simplification of the import process of 3D cell component models or whole cells extracted from 3D Microscopy (Section 2.2).

The export and import capabilities will be expanded to support *SBML* in the future. The advantage of the implemented 2D View (Section 2.4.2) is that networks and layouts created by other applications may be imported into *CmPI* and localized and explored in 3D space.

The cell environment will be extended in different directions: The *CellEditor* will provide the possibility of building cell models of different species to be used in other applications as well, while the *CellExplorer* will be advanced to an interactive learning environment which will offer a way to give students a spatial idea of the cell.

Another advantage of 3D visualizations is the ability to show the realistic 3D structure of components. Therefore, the integration of the molecular level is intended, correlated with organelles as well as networks. Combined with capabilities to simulate these networks, this framework would be another interesting contribution to Synthetic Biology.

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