Experimental Design and Analysis for Human-Subject Visualization Experiments

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Abstract—**Experimental design is important, but is often under-supported, in systems biology research. To improve experimental design, we extend the visualization of complex sphingolipid pathways to study biosynthetic origin in SphinGOMAP. We use the ganglio-series sphingolipid dataset as a test bed and the Java Universal Network / Graph Framework (JUNG) visualization toolkit. The result is an interactive visualization tool and data model for experimental design in lipid systems biology research. We improve the current SphinGOMAP in terms of interactive visualization by allowing (i) choice of four different network layouts, (ii)dynamic addition / deletion of on-screen molecules and (iii) mouse-over to reveal detailed molecule data. Future work will focus on integrating various lipid-relevant data systematically *i.e.* SphinGOMAP biosynthetic data, Lipid Bank molecular data (Japan) and Lipid MAPS metabolic pathway data (USA). We aim to build a comprehensive and interactive communication platform to improve experimental design for scientists globally in high-throughput lipid systems biology research.**

Keywords—Experimental design, graph layout algorithms, interactive visualization, sphingolipid pathways

I. Introduction

EXPERIMENTAL design is an important, but often undersupported aspect in systems biology research. On one hand, high-throughput technologies such as DNA microarrays and mass spectrometry are continually advanced to improve the collection of high-resolution quantitative and temporal data. On the other hand, computational science is evolving in tandem to produce a diverse spectrum of bioinformatics tools and algorithms to analyze increasingvolumes of data post-experiment. In this paradigm, there arelimited resources to aid the design of large-scaleexperiments that involve systematically collectingheterogeneous data from complex biological systems oversustained periods. Thorough and informed experimental design is critical for successful systems biology research. Because of technological and analytical advancements, there is an increasing momentum shift from studying biological systems in relative isolation to high-throughput systems biology research. Where investigators could adequately articulate and design experiments by hand in the paradigm of single-variable experiments, they now require computational tools to visualize and handle multi-variable experiments. Consequently, a measure of good experimental design may be how well it reduces computational load during data analysis post-experiment. In other words, the computational load for a healthy and complete research process is balanced throughout experimental design, data acquisition and post-experiment analysis.

Such experimental design can be achieved by increasing the quantity and quality of *a priori* knowledge available. With increasing efforts for community annotation and sharing of scientific data, it is certain that researchers have no lack of *a priori* data if they know where to look. On the other hand, the quality of such knowledge is not guaranteed *i.e.* researchers may not know how to look. For the purpose of ensuring both quantity and quality, multiple standards have been proposed for various biological data [1-4].

To improve the quality of *a priori* knowledge, we propose an interactive visualization data model for experimental design in systems biology, using the ganglio-series of sphingolipids dataset as a test bed. We extend and improve the current SphinGOMAP [5] in terms of interactive visualization by implementing (i) dynamic network visualization, (ii) choice of four different network layouts and (iii) mouse-over to reveal detailed molecule data. These features enhance user experience in dealing with highvolume, large-scale systems biology data.

II. METHODS

We design and implement our interactive visualization tool based on molecular data of sphingolipids from SphinGOMAP [5] and programmatic visualization library from JUNG [6]. Furthermore, we extend our tool to include a data model for potential interactions with existing lipid resources such as LipidBank [7] and Lipid MAPS [8].

A. SphinGOMAP [5]

SphinGOMAP is a pathway map to organize and visualize sphingolipids. The expressed objective of SphinGOMAP is to "promote dialog about the 'knowns' and 'unknowns' ofsphingolipid biosynthesis and lead to experiments to refine this model"[5]. Thus, SphinGOMAP is an active document that evolves with emerging scientific discovery.

The current SphinGOMAP can be improved in terms of interaction and visualization. First, from the SphinGOMAP website, release 2.0 in October 2007 displays ~450 compounds in a static file as a Microsoft PowerPoint slide or JPEG image. In its present form, the static images do not allow users to interact freely with the map. Second, furthermore, the various families (series) of sphingolipids have to be presented separately in different files. This is because the density and scale of sphingolipid networks is too large to allow a panoramic view and yet provide sufficient detail at the same time. Third, the data in SphinGOMAP contains only molecular structure, category, common name and LipidBank ID. Thus, the connectivity of the pathway map is compromised for clarity and some detail.

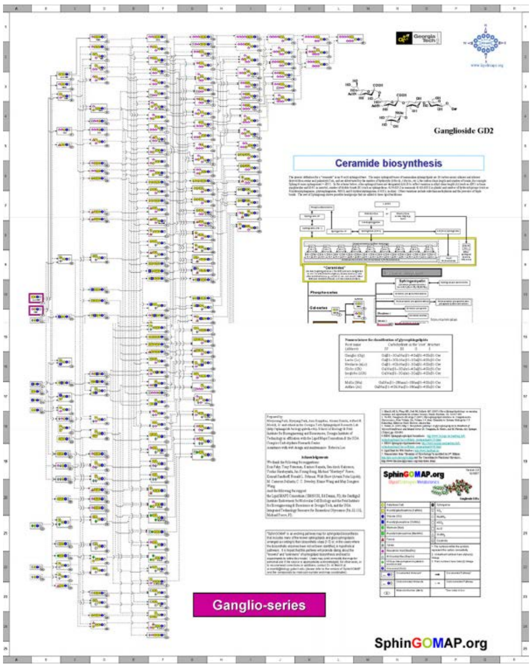


Fig. 1. Current visualization of the ganglio-series of sphingolipids in SphinGOMAP [5]. This is available as a Microsoft PowerPoint slide or static JPEG image file that allows limited interactivity and compromises map connectivity for clarity and some detail.

On the other hand, a simple and intuitive legend to denote biochemical functional groups for sphingolipids and its derivatives is deployed in SphinGOMAP. The legend helps users recognize distinct structural moieties at a glance. This is especially helpful to determine recurring patterns in biosynthetic inheritance.

We address these problems with interaction, data separation, and limited detail using a data model and software tool. We focus on the ganglio-series of sphingolipids from SphinGOMAP as our test data. This software tool is implemented using an open-source visualization toolkit.

B. JUNG - Java Universal Network/Graph Framework[6]

The JUNG API for Network/Graph visualization provides common and extensible software library for analysis and visualization of data that can be represented as a graph or network. In this work, we integrate the Kamada-Kawai [9], Fruchterman-Reingold [10], ISOMLayout [11-12] and CircleLayout algorithms provided by the JUNG software library. We use the JAVA graphics API to render the structure of the molecules.

The vertex of molecules is generated based on the internal chain structures. These chains are generated *a priori* during database filtering. In database filtering, we extract the molecular structure as formulas and reorder them based on the cardinality of molecules in a chain. Starting from a shorter chain, the derivatives are generated and mapped to its parent.

An in-memory tree is built in two steps to generate this type of hierarchy. In the first step, we select single-chain molecules to generate the basic hierarchy, while in the second step, all molecules that have branches are broken into different chains. For instance, a molecule "Galb 13GalNAcb 1-4(NeuAca2-3)Galb 1-4GlcCer" (b – beta), is broken down into two chains, Galb 1-3GalNAcb 1-4Galb 14GlcCer and NeuAca 2-3Galb 1-4GlcCer. We search for these two chains throughout the basic hierarchy built in the first phase to extract the parent chain. Note that every branched molecule will have more than one parent.

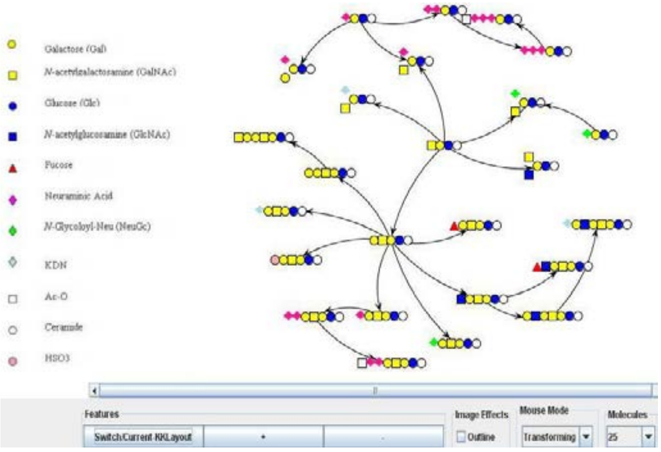
Once this relationship is built, it is sent as an input (Hashtable structure) to the visualization module. The visualization module extracts the molecular structure and renders it as an image on a label, which acts as a vertex for the graph structure.

C. LipidBank [7] *and Lipid MAPS* [8]

LipidBank and Lipid MAPS are global leading sources for lipid data that originated from Japan and USA respectively. On one hand, LipidBank contains primary data for an extensive number of lipids (~6000 molecules) in terms of molecular structure, scientific and common names, spectral information and literature. On the other hand, Lipid MAPS is focused on more secondary data in terms of lipid interactions within mammalian cells by "characterizing the global changes in lipid metabolites ('lipidomics')"[7]. Lipid MAPS contains not only structural data and annotations of biologically active lipid molecules, information about lipid metabolic pathways, experimental protocols, standards and time-course results are also available. Lipid MAPS is also linked to public databases for relevant molecules such as lipid-associated proteins.

III. Results

An overview of the visualization is presented in Figure 2. In this section, we report noteworthy features such as (a) dynamic network visualization with increasing complexity, (b) choice of different network layouts and (c) mouse-over to reveal detailed molecule data. The intuitive legend from SphinGOMAP for describing biochemical functional groups on the sphingolipid molecules is preserved in our visualization.



Mouse Mode: Transforming

Molecules: 25

Fig. 2. Screen capture of interactive visualization of sphingolipid ganglio-series (25 molecules selected). Noteworthy features include spontaneous addition / deletion of molecules with increasing network complexity,

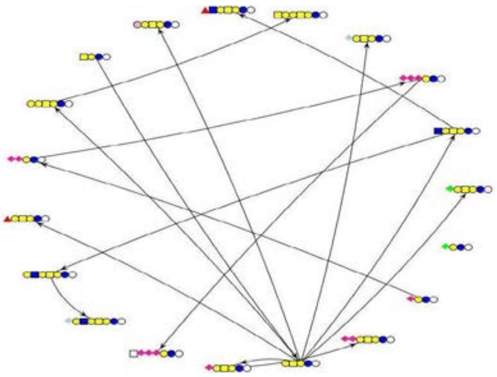
choice of four different network layouts, and listing of detailed molecule data on mouse-over.

A. Dynamic network visualization with increasing complexity

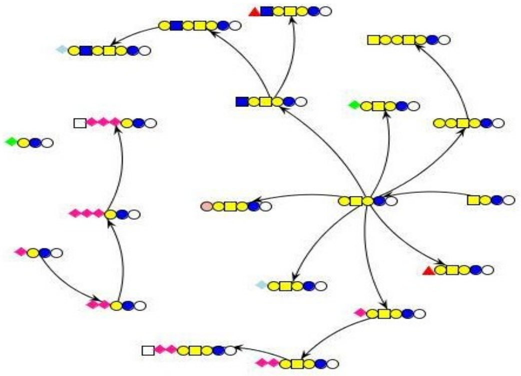
The complexity of the networks is visualized "on-the-fly" so users may add or delete molecules for visualization spontaneously. To do this, all molecules are filtered from the database *a priori*. We derive the parent-derivative relationship between molecules as a hierarchical structure in memory, starting from the smallest chain to cover all molecules of the given series within the SphinGOMAP database. Thus, molecules may be added or deleted spontaneously in a pre-determined hierarchical order *i.e.* ordered sequence.

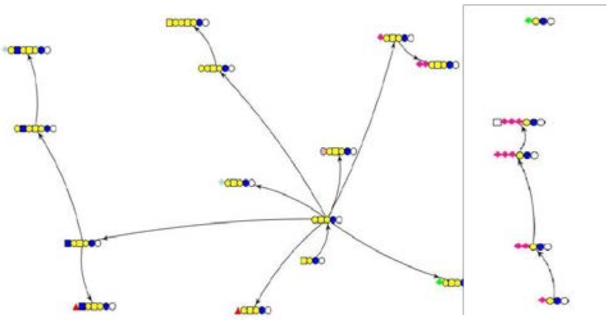
B. Choice of network layouts

We implement a choice of four different network layouts as seen in Figure 3. Of the four layout algorithms used, the Kamada-Kawai algorithm was most comprehensible even when the network complexity grew beyond 25 molecules. Circle layout works well with smaller numbers of molecules but becomes more complicated with a much larger number of molecules, for instance, 60 molecules.



(a) Circle





(b) Kamad-Kawai (KK)

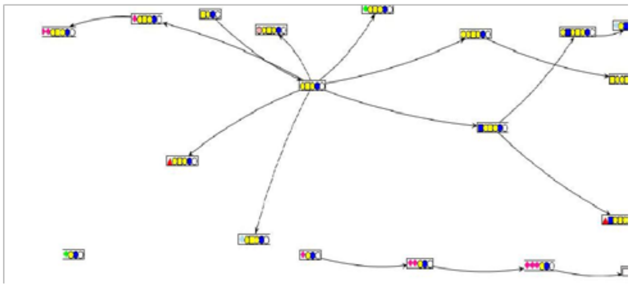


Fig. 3. Circle (a), Kamad-Kawai (b), ISOM (c), and FruchtermanReingold (d) layouts of 25 molecules. Different layouts reveal different network features that may lead users to identify and implement suitable alterations in experimental design.

(d) Fruchterman-Reingold (FR)

The self organizing graph layout algorithm (ISOM) renders the graph more widely using as much space as it needs for specifically rendering each relationship. As a result, the network structure becomes clearer, but less manageable. The Fruchterman-Reingold (FR) layout is similar to the ISOM layout and works fine with 25 molecules when the maximum edge length is smaller than 5 cm on a screen. However, it becomes unwieldy, in terms of providing a panoramic view, with a much larger number of molecules. Thus, different layouts may reveal different network features depending on the level of network resolution that users may desire.

C. Mouse-over

The mouse-over feature, presented in Figure 4, allows users to view detailed molecule data: common name, chemical category and Lipid Bank ID. Using this feature to incorporate more detailed sphingolipid molecule information from Lipid Bank and Lipid MAPS, we are eager to link biosynthetic network data from SphinGOMAP with primary and secondary data.

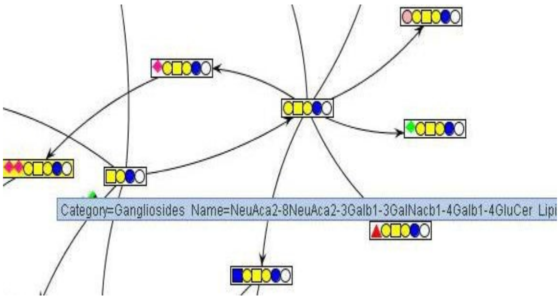

Category=Gangliosides Name=NeuAca2-8NeuAca2-3Galb1-3GalNacb1-4Galb1-4GluCer Lipi

Fig. 4. Zoomed image of mouse-over feature. Detailed molecule data is revealed; this feature allows data from Lipid Bank and Lipid MAPS to be incorporated and linked for a more comprehensive interaction with specific sphingolipid molecules and pathways.

IV. Discussion

Collecting large-scale biological network data is not trivial, especially as immeasurable effort and resources are, and will continue to be, invested in performing experiments and gathering observations. Thus, data organization must rightfully receive equal emphasis, if not more, with the increasing focus on systems biology enabled by highthroughput technology.

To facilitate experimental design for systems biology research, we extend and improve the current SphinGOMAP in terms of interactive visualization. We do this by implementing (i) dynamic network visualization, (ii) choice of four different network layouts and (iii) mouse-over to reveal detailed molecule data. These features enhance user experience in dealing with high-volume, large-scale systems biology data. Thus, this work contributes to systems biology research by improving visualization, interactivity and usability of massive, complex biological networks.

Specifically for experimental design, a typical user may use our tool: (a) based on the feature of dynamic network visualization, to track the biosynthetic origins of a specific molecule, or to place it in the wider context of downstream derivatives or parallel molecules, i.e. "cousins", in terms of chemical inheritance; (b) to reveal, or confirm, previously unrecognized biosynthetic relations, using the various different network layouts, and (c) to examine / juxtapose research findings with the knowledge of metabolic data that could account for differences between experimental data and *a priori* network models by linking with Lipid MAPS.

Our implementation represents concrete improvement from previous visualizations in terms of data organization and representation built on software tools.

Using this tool, we aim to close the loop for information flow in terms of directing *a priori* knowledge and community feedback from past experiments into better experimental design. Future work will focus on increasing the data collaboration between SphinGOMAP, Lipid Bank and Lipid MAPS. By linking biosynthetic origin data, molecular data and metabolic pathway data, scientists can look forward to a synergistic interaction with more aspects of lipid molecule information.

ACKNOWLEDGMENT

The authors thank Christopher Haynes, Mitchell Parry Jeremy Allegood and Todd Stokes for technical assistance and discussions; Lipid MAPS for the use of sphingolipid biosynthetic data via SphinGOMAP.

系统生物学中用于实验设计的交互式可视化工具和数据模型

_摘要_ - 在系统生物学研究中实验设计十分重要，但往往得不到支持。为了改进实验设计，我们扩展了复杂鞘脂途径的可视化，以研究 SphinGOMAP 中的生物合成起源。我们使用 ganglio 系列鞘脂数据集作为测试平台，使用了 Java 通用网络/图形框架（JUNG）可视化工具包。我们的成果是一个交互式可视化工具和数据模型，用于脂质系统生物学研究的实验设计。通过允许 (i) 可选四种不同网络布局，(ii) 动态添加/删除屏幕上的分子和 (iii) 鼠标悬停以显示详细的分子数据，我们改进了当前 SphinGOMAP 在交互式可视化方面的性能。未来的工作将集中在系统地集成各种脂质相关数据，即 SphinGOMAP 生物合成数据、Lipid Bank 分子数据（日本）和 Lipid MAPS 代谢途径数据（美国）。我们的目标是建立一个全面的交互式交流平台，以改良全球科学家在高通量脂质体系生物学研究中的实验设计。

关键词 - 实验设计，图形布局算法，交互式可视化，鞘脂途径

I. 引言

实验设计是系统生物学研究中一个重要的但常常得不到支持的方面。 一方面，随着 DNA 微阵列和质谱等高通量技术不断推进，对高分辨率定量和时间数据的采集得以改进。另一方面，计算科学正在不断发展，产生了各种各样的生物信息学工具和算法，以便分析日渐增长的实验后数据量。在这个范例中，有限的资源有助于设计大规模的实验，这些实验涉及系统地采集持续时间内来自复杂生物系统的异质数据。

周密而明智的实验设计对于成功的系统生物学研究至关重要。由于技术和分析的进步，从研究相对独立的生物系统转向研究高通量系统生物学的势头正在不断增长。研究员能够在单变量实验的范例中清楚地人工阐述、设计实验，然而对于多变量实验，他们现在需要计算工具来进行可视化和处理。因此，一个实验设计的优劣，可以根据在分析实验后数据的过程中，它多大程度地减少计算负荷来评估。换句话说，健全完整的研究过程中，计算负载在整个实验设计、数据采集和实验后分析过程中保持平衡。

这种实验设计可以通过增加可用的先验知识的数量和质量来实现。随着社区标注和科学数据共享的力度不断加大，如果研究人员知道在哪里查找，那他们肯定不缺乏先验数据。但是，这种知识的质量并不能保证，即研究人员可能不知道如何看待。为了确保数量和质量，已经为各种生物学数据提出了多种标准。

为了提高先验知识的质量，我们提出了一个用于系统生物学实验设计的交互式可视化数据模型，使用 ganglio 系列鞘脂数据集的作为测试平台。通过实现 (i) 动态网络可视化，(ii) 可选四种不同网络布局和 (iii) 鼠标悬停以显示详细的分子数据，我们在交互式可视化方面扩展和改进了当前的 SphinGOMAP。这些功能提高了处理高容量、大规模系统生物学数据的用户体验。

II. 方法

基于来自 SphinGOMAP 的鞘脂分子数据和 JUNG 的程序化可视化库，我们设计和实现了交互式可视化工具。此外，我们扩展了我们的工具，来囊括与现有脂质资源（如 Lipid Bank 和 Lipid MAPS）有潜在相互作用的数据模型。

A. SphinGOMAP

SphinGOMAP 是用于组织和可视化鞘脂的途径图。SphinGOMAP 的明确目标是“促进关于神经鞘脂生物合成的'已知'和'未知'的对话并引导实验来完善这个模型”。 因此，SphinGOMAP 是随着新兴科学发现而发展的活跃文档。

当前的 SphinGOMAP 可以在交互和可视化方面进行改进。第一，SphinGOMAP 网站在 2007 年 10 月发布 2.0 版将静态文件中的大约 450 个化合物显示为微软 ppt 或 JPEG 图像。在目前的形式下，静态图像不允许用户与途径图自由交互。 第二，各种鞘脂类家族（系列）必须分别在不同的文件中列出。这是因为鞘脂网络的密度和规模太大，无法在观察全景的同时又提供足够的细节。第三，SphinGOMAP 中的数据仅包含分子结构、类别、通用名称和 Lipid Bank ID。因此，路径图为了清晰度和一些细节牺牲了连通性。

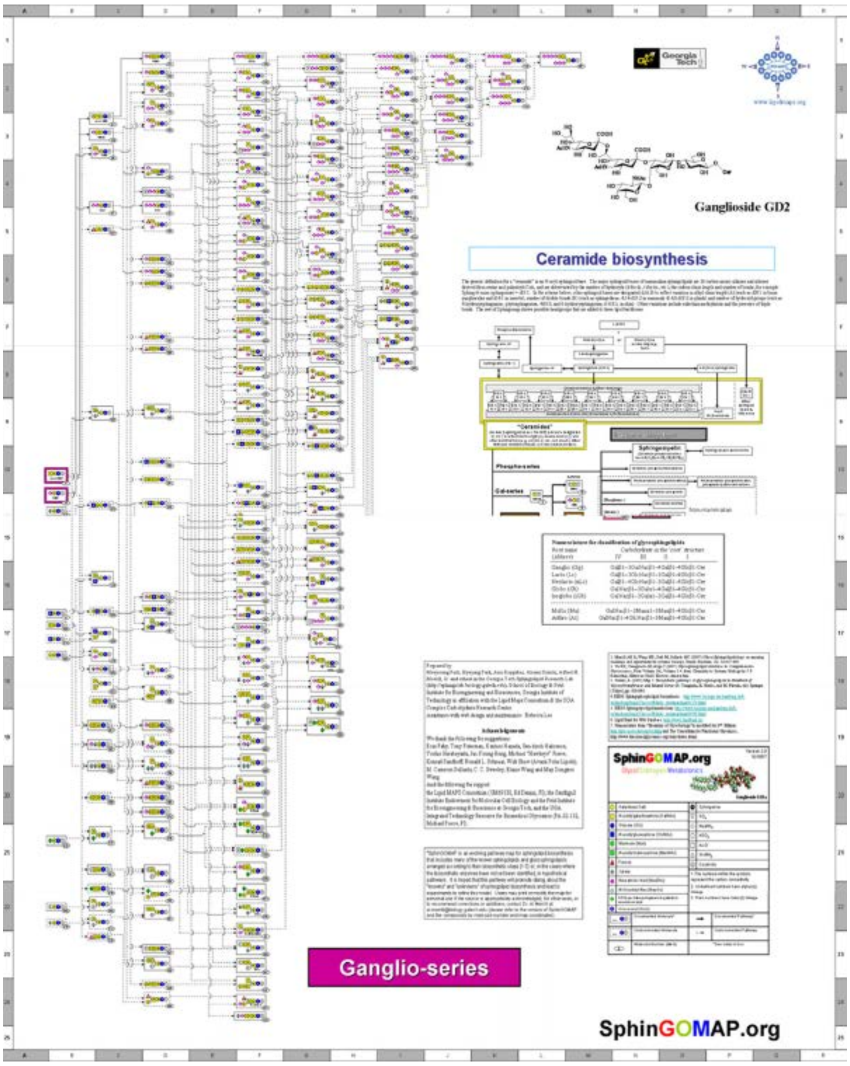


图 1. 目前 SphinGOMAP 中 ganglio 系列神经鞘脂的可视化结果。这可以作为微软 ppt 或静态 JPEG 图像文件提供，它交互性有限，并且为了清晰度和一些细节牺牲了连通性。

另一方面，在 SphinGOMAP 中部署了一个简单而直观的图例，用于表示鞘脂及其衍生物的生化官能团。该图例帮助用户一目了然识别不同的结构部分。这对于确定生物合成遗传中的递归模式特别有用。

使用数据模型和软件工具，我们通过交互、数据分离和有限的细节来解决这些问题。我们专注于 SphinGOMAP 的 ganglio 系列鞘脂作为我们的测试数据。该软件工具由开源可视化工具包实现。

B. JUNG - Java 通用网络/图形框架

用于网络/图形可视化的 JUNG API 提供通用的可扩展软件库，来对可以表示为图形或网络的数据进行分析和可视化。在这项工作中，我们整合了由 JUNG 软件库提供的 Kamada-Kawai、Fruchterman-Reingold、ISOMLayout 和 CircleLayout 算法，用 Java 图形 API 来渲染分子的结构。

分子的顶点是基于内部链结构生成的。这些链是在数据库过滤期间先验产生的。在数据库过滤中，我们根据公式提取分子结构，并根据链中分子的基数对它们进行重新排序。从较短的链开始，生成衍生物并将其映射到其父项。

分两步在内存中构造一棵树，来生成这种层次结构。第一步，我们选择单链分子来生成基本层次结构，第二步，将所有具有分支的分子分解成不同的链。例如，分子“Galb 1-3 GalNAcb 1-4(NeuAca2-3)Galb 1-4GlcCer”(b-beta) 分解成两条链，Galb 1-3 GalNAcb 1-4 Galb 14 GlcCer 和 NeuAca 2-3 Galb 1-4 GlcCer。我们在第一步里构建的基本层次结构中搜索这两个链，以提取父链。 请注意，每个分支分子有多个父亲。

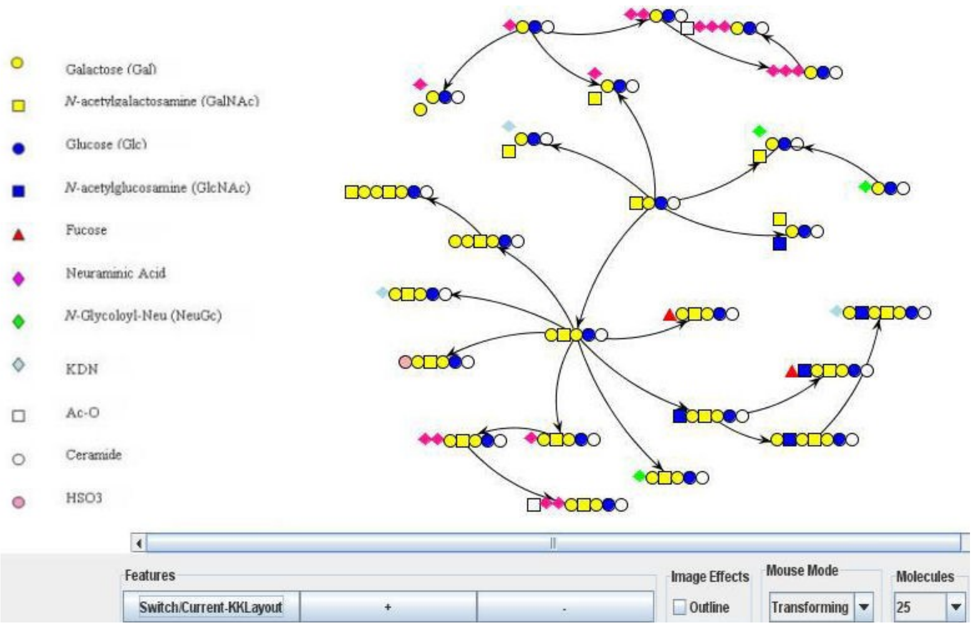
一旦建立了这种关系，它将作为输入（哈希表结构）发送到可视化模块。可视化模块提取分子结构并作为图形在标签上渲染，该标签充当图结构的顶点。

C. Lipid Bank 和 Lipid MAPS

Lipid Bank 和 Lipid MAPS 是脂质数据的全球主要来源，分别来自日本和美国。一方面，在分子结构、科学通用的名称、光谱信息和文献方面，Lipid Bank 包含大量脂质（约 6000 个分子）的一手数据。另一方面，通过“表征脂质代谢物的全球变化（'脂质组学'）”，Lipid MAPS 侧重于更多的哺乳动物细胞内脂质相互作用方面的二手数据。Lipid MAPS 不仅包含生物活性脂质分子的结构数据和标注，还提供关于脂质代谢途径、实验方案、标准和时间过程结果的有关信息。Lipid MAPS 链接到相关分子如脂质相关蛋白的公共数据库。

III. 结果

图 2 给出了可视化的概述。在本节中，我们展示了一些值得注意的特性，例如 (a) 随着复杂度增加的动态网络可视化， (b) 可选不同网络布局和 (c) 鼠标悬停以显示详细分子数据。我们将 SphinGOMAP 中用于描述鞘脂分子上生物化学官能团的直观图例保留在可视化中。


● Galactose (Gal)
■ N-acetylgalactosamine (GalNAc)
● Glucose (Glc)
■ N-acetylglucosamine (GlcNAc)
▲ Fucose
◆ Neuraminic Acid
◆ N-Glycoloyl-Neu (NeuGc)
◇ KDN
□ Ac-O
○ Ceramide
● HSO3
+
-

Features: Switch/Current-KKLayout

Image Effects: □ Outline

Mouse Mode: Transforming

Molecules: 25

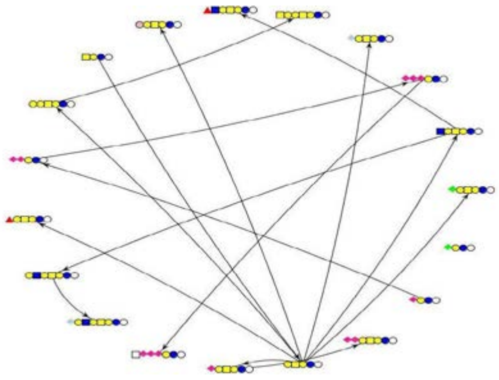
图 2. ganglio 系列鞘脂（选中 25 个分子）的交互式可视化的截屏。值得注意的功能包括随着网络复杂度的增加自发添加/删除分子、可选四种不同的网络布局，以及在鼠标悬停时列出详细的分子数据。

A. 随着复杂度增加的动态网络可视化

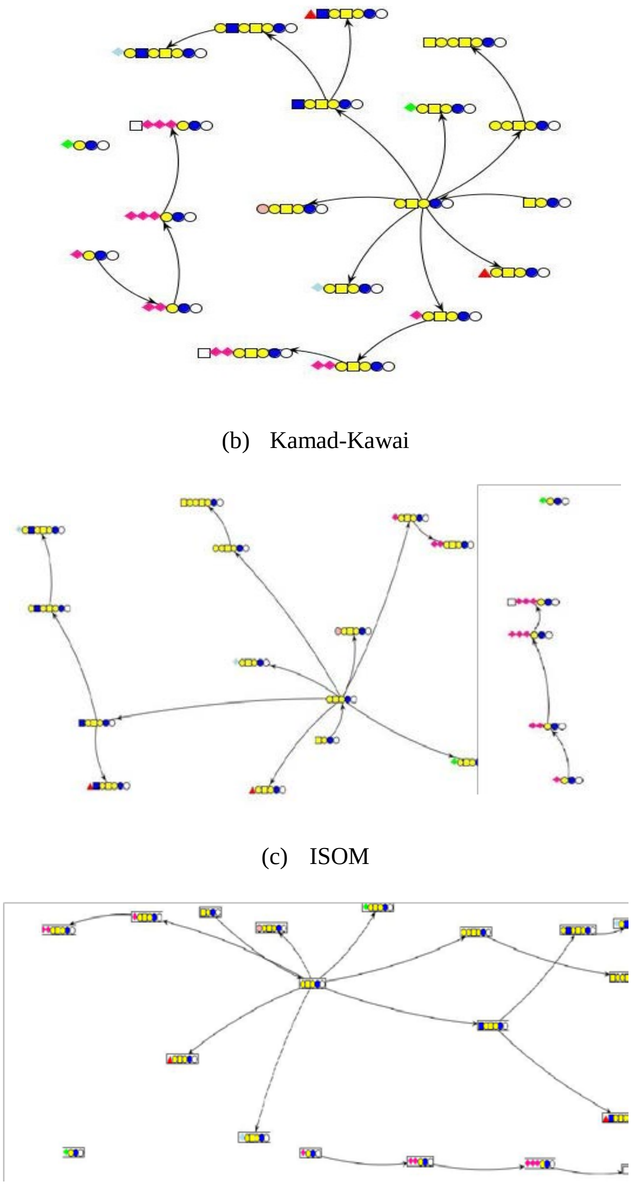
网络的复杂性是“实时”可视化的，因此用户可以自发地添加或删除可视化分子。为了做到这一点，所有的分子都会事先从数据库中过滤掉。我们推导出分子之间的父子关系，来作为内存中的层次结构，从最小的链开始，覆盖 SphinGOMAP 数据库中给定序列的所有分子。因此，分子可以以预定的等级顺序即有序序列自发地添加或删除。

B. 网络布局选择

我们实现了四种不同网络布局的选择，如图 3 所示。在使用的四种布局算法中，Kamada-Kawai 算法是最易懂的，即使网络复杂度超过了 25 个分子。CircleLayout 适用于分子数量较少时，但在分子数量多时（例如 60 个分子）变得更复杂。



(a) Circle



(b) Kamad-Kawai

(c) ISOM

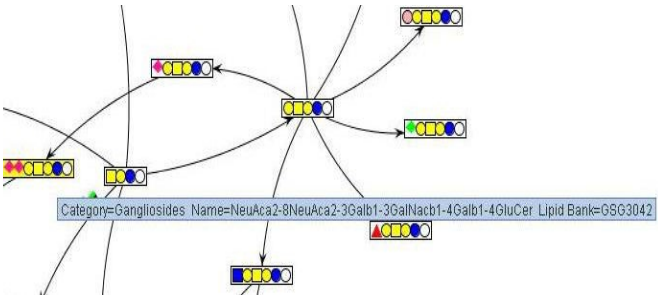
(d) Fruchterman-Reingold

图 3. 25 个分子时的 Circle (a), Kamad-Kawai (b), ISOM (c) 和 Fruchterman-Reingold (d) 布局。不同的布局显示不同的网络特性，这些特性可能导致用户识别和进行适当的实验设计更改。

自组织图形布局算法 (ISOM) 使用尽可能多的空间来渲染图形，以便专门呈现每个关系。结果网络结构变得更清晰，但更加不可控。 Fruchterman-Reingold (FR) 布局与 ISOM 布局类似，当屏幕上有 25 个分子，且最大边长小于 5 厘米时，效果不错。然而，在分子数量变多时，无法提供全景视图。因此，根据用户可能需要的网络分辨率级别，不同的布局可能会呈现不同的网络特性。

C. 鼠标悬停

图 4 所示的鼠标悬停功能允许用户查看详细的分子数据：通用名称、化学类别和 Lipid Bank ID。用这一功能将 Lipid Bank 和 Lipid MAPS 中更详细的鞘脂分子信息整合在一起，我们将 SphinGOMAP 的生物合成网络数据与一手数据和二手数据链接起来。



Category=Gangliosides Name=NeuAca2-8NeuAca2-3Galb1-3GalNacb1-4Galb1-4GluCer Lipid Bank=GSG3042

图 4. 鼠标悬停功能的放大图像。展示了详细的分子数据被；该功能将 Lipid Bank 和 Lipid MAPS 的数据合并链接，以便与特定的鞘脂分子和途径进行更全面的交互。

IV. 讨论

采集大规模的生物网络数据是很重要的，尤其是现已投入了难以估量的工作量和资源来进行实验和采集观测结果，且还将继续投入。因此，随着高通量技术带来的系统生物学越来越受关注，我们必须合理地对数据组织给予同等重视。

为了便于系统生物学研究的实验设计，我们在交互式可视化方面扩展并改进了当前的 SphinGOMAP。通过实现 (i) 动态网络可视化，(ii) 四种不同网络布局的选择以及 (iii) 鼠标悬停来显示详细的分子数据，我们实现了这一点。这些特性提高了处理大批量、大规模系统生物学数据的用户体验。因此，这项工作通过改善大规模复杂生物网络的可视化、交互性和可用性来促进系统生物学研究。

特别是对于实验设计，典型用户这样可以使用我们的工具： (a) 基于动态网络可视化的特性，追踪特定分子的生物合成起源，或将其置于更广泛的下游衍生物或平行分子（化学遗传方面的“堂兄弟”）的环境中; (b) 用各种不同的网络布局来显示或确认先前未识别的生物合成关系，以及 (c) 对有代谢数据知识的研究结果进行检查/并置，这些结果通过与 Lipid MAPS 连接，可以解释实验数据与先验网络模型之间的差异。

我们的实现体现了对以前的可视化在数据组织基于软件工具的呈现方面的具体改进。使用此工具，我们旨在停止信息流的循环，将先验知识和来自过去实验的社区反馈转化为更好的实验设计。未来的工作将集中在增加 SphinGOMAP、Lipid Bank 和 Lipid MAPS 之间的数据协作。通过连接生物合成起源数据、分子数据和代谢途径数据，科学家可以期待与脂质分子信息的更多方面产生协同交互。

致谢

作者感谢 Christopher Haynes，Mitchell Parry Jeremy Allegood 和 Todd Stokes 提供技术援助和讨论；感谢 Lipid MAPS 允许通过 SphinGOMAP 使用鞘脂生物合成数据。