Proceedings of the 26th Annual International Conference of the IEEE EMBS
San Francisco, CA, USA • September 1-5, 2004

Dynamic Pathway Modeling of Sphingolipid Metabolism

Peter A. Henning[1], Alfred H. Merrill, Jr.[2*], May D. Wang[1*]

1Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University
2School of Biology, Georgia Institute of Technology
313 Ferst Drive, Atlanta, GA 30332, USA

ABSTRACT

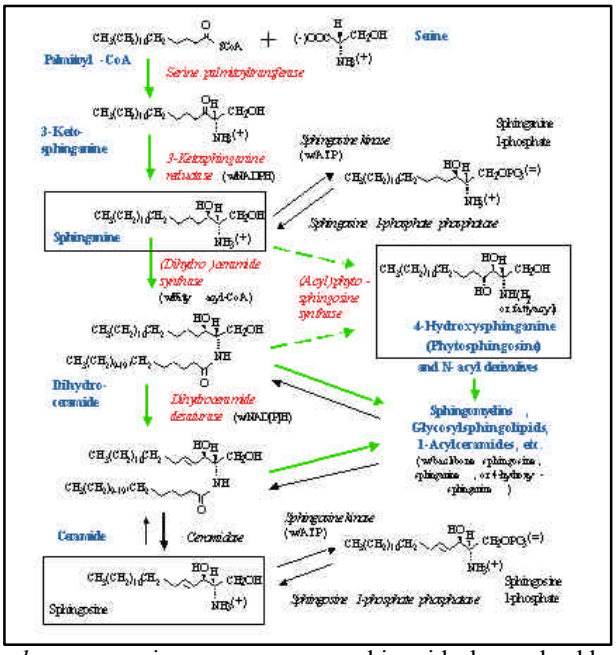
In this paper, we report our research results on computational metabolome study.

1. INTRODUCTION

Tremendous advances in molecular biology, both in understanding and developing of high throughput data acquisition techniques such as mass spectrometry, provide ample support for studying complex biological control networks [2, 3]. Even with recent substantial advances in data management, genomics, and robotics, the discovery of new pharmaceutical agents has not accelerated over the last few years. Although the drug industry may approach a saturation point for single targeted drugs, simple drug treatments that effectively target a control network still hold a great deal of promise[4]. Mathematical simulations of these complex networks provide researchers a means of linking the biochemistry of a reaction pathway to not only the resulting healthy phenotype but also to the dysfunctional disease state[5-7]. While still in its early years, the principles of Systems Biology could have profound effects leading to more hypothesis-driven research in drug discovery [8] and other medical treatments.

2. OVERVIEW OF SPHINGOLIPID STUDY

Sphingolipids perform a wide variety of biological functions: formation of specialized structures, participation in cell-cell and cell-substratum interactions, modulation of the behavior of cellular proteins and receptors, and signal transduction both as extracellular agonists and intracellular mediators. They are synthesized



de novo via a common sphingoid base backbone (sphinganine) that is modified to produce ceramides and more complex phospho- and glycosphingolipids, some of which are covalently attached to proteins[1]. The many known functions with complex undetermined consequences make sphingolipid pathways an excellent choice to study with new modeling techniques such as system approaches

3. SYSTEM DEVELOPMENT

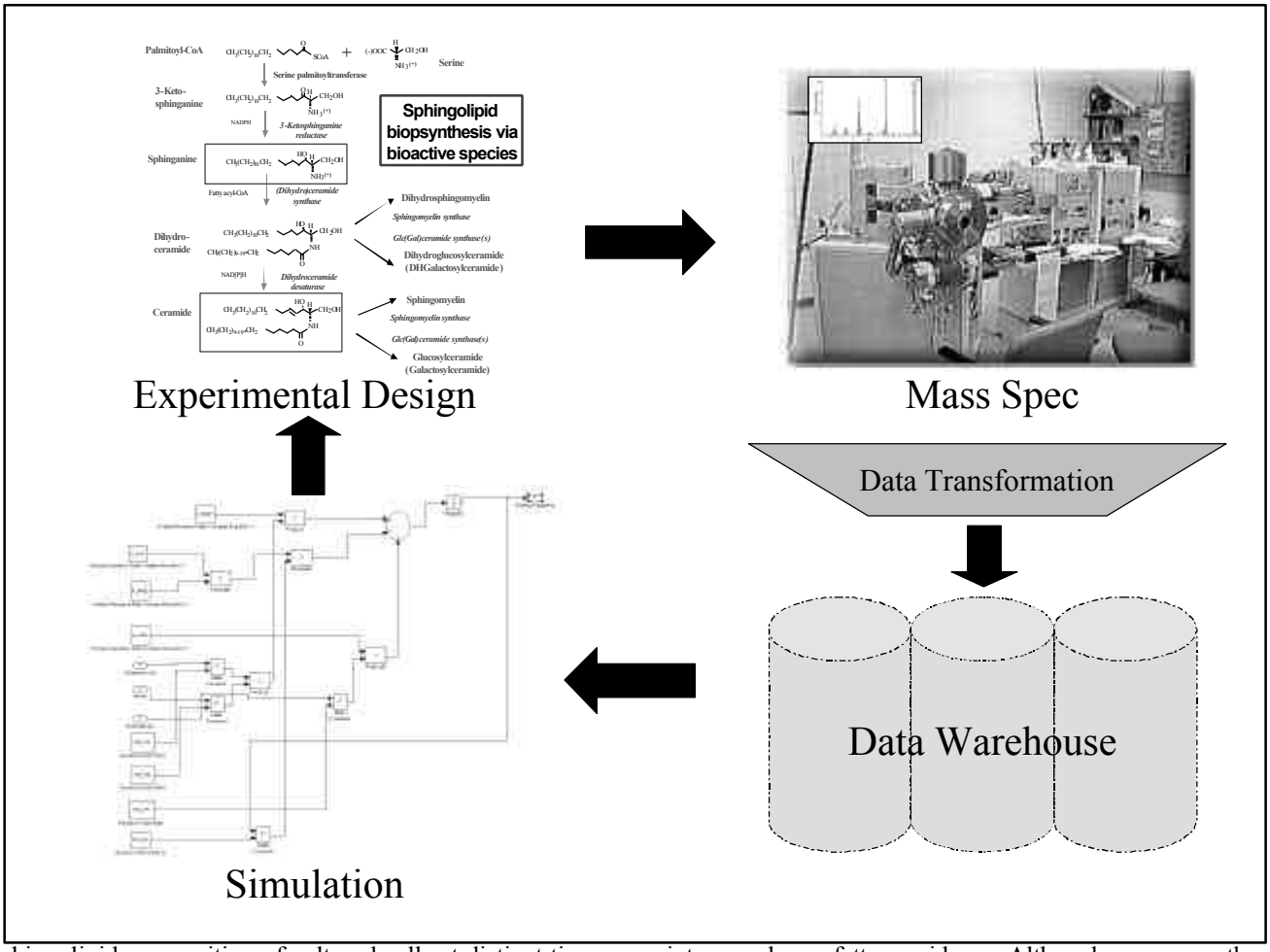
The goal of this research is to develop system approach for biology discovery. Specifically, we want to quantitatively characterize the sphingolipid metabolism pathway in order to understand their roles in normal physiological processes and investigate possible treatment

[1] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 313 Ferst Dr., Atlanta, Georgia, USA. Email: maywang@bme.gatech.edu
[2] School of Biology, Georgia Institute of Technology, 315 Ferst Dr., Atlanta, Georgia, USA. Email: al.merrill@biology.gatech.edu
*Authors to which correspondence should be addressed.

options for a variety of diseases including cancer. As shown in Figure 1, first, the latest proposed sphingolipid metabolism pathways are carefully examined and evaluated to insure adequate experimental design. The focus then shifts to developing optimized mass spectrometry protocols to obtain accurate measurements of the

4. IMPLEMENTATION

A six-node network was constructed using actual sphingolipid metabolism data obtained using mass spectrometry. All of the data points concerned the movement of various sphingolipds that contained a



sphingolipid composition of cultured cells at distinct time points. After the data is collected, the experimental results are stored in a database and are managed by DBMS. The governing system equations are formed, and the parameters are identified through a series of regression sequences. Then a time-based dynamical simulation is created. The simulation offers powerful insight into the mysterious nature of the biological pathway and initiates an iterative process, in which new hypotheses can be quickly formulated and then validated at the bench. Because of the size and complexity of this pathway, 3-D visualization tools are designed and implemented to enhance understanding of the biological process and to reformulate hypotheses.

sixteen-carbon fatty acid. Although many other sphingolipid concentrations were also included in the utilized data set, none had the dimensionality required to prepare a larger network. The image below depicts the network configuration taken from a SIMULINK model file. The six species are dihydroceramide, dihydroglucosylceramide, dihydrosphingomyelin, ceramide, glucosylceramide, and sphingomyelin represented respectively as DH, GCDH, SMDH, C, GC, and SM.

Each node in the network model presented previously is constructed from a single differential equation that describes the accumulation of the particular species with respect to time. Each node in the system has a different number of dependencies on other nodes as evidenced in

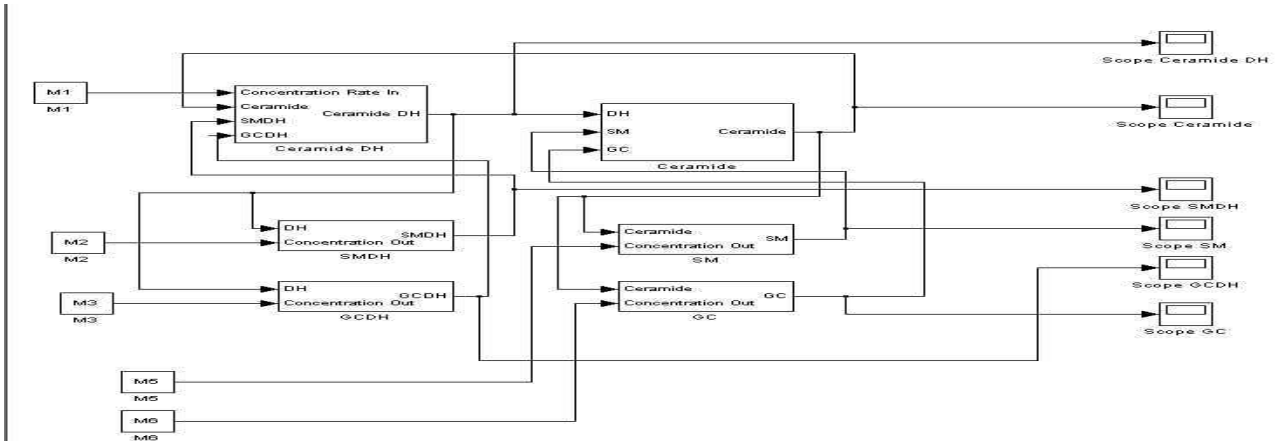


figure 1. An example of the dependence can be seen when comparing the Ceramide Node with the GCDH node. The Ceramide node is dependent on the relative amount of DH, SM, and GC whereas the GCDH node is dependent only on the DH concentration and any mass transfer of the species entering and leaving the system. It should be noted that because each of these reactions is modeled as reversible there is an intrinsic dependence of the node on itself. This dependence for Ceramide is shown in the figure below. Notice the feedback loop, shaded gray on image, branching off of output coming out of the integrator.

The system can be modeled using many chemical reaction schemes, which have well developed systems equations used in control including simple models such a continuously stirred reactor. $\frac{dV}{dt} = F_i - F$

$$\frac{dC_A}{dt} = \frac{F_i}{V}(C_{Ai} - C_A) + k_r C_B^b - k_f C_A^a$$

$$\frac{dC_B}{dt} = \frac{F_i}{V}(C_{Bi} - C_B) + k_f C_A^a - k_r C_B^b$$

where: V = reactor volume, F_i = inlet volumetric flow rate, F = outlet volumetric flow rate, C_A = concentration of species A in the reactor and exiting the reactor, C_{Ai} = concentration of species A in the entrance stream, C_B = concentration of species B in the reactor and exiting the reactor, C_{Bi} = concentration of species B in the entrance stream, k_r = reverse reaction rate constan, k_f = forward reaction rate constant, a = reaction order of species A, β = reaction order of species B

Although well defined in a CSTR, the mass flux for an organic biochemical reactor is rather unclear due to its ability to support active transport. The constants V and F_i, which correspond to the effective volume of the cell culture reactor and inlet volumetric flow rate, cannot be quantified efficiently. The system is also biologically incomplete and greatly under sampled so the mass flux parameter in the species accumulation differential equations has been represented with a single constant value M_x, which can be either positive or negative representing net influx or outflow of the species in the system.

Enzyme concentration and other properties of enzymes has long been known to be a major player in biochemical reactions so it may seem ludicrous to build a network that is built of pure mass action parts. There is very little hope that a pure mass action driven network can complete classify a biological network, nevertheless mass action models are a starting point that should allow the foundation of various computational techniques that can be scaled up once more data becomes available.

Although the parameters of each species accumulation function can be solved independently when given enough measurements, the equations are inherently dependent on each other as indicated by network diagram in the first section.

Linear Regression, Gauss-Newton Method, Marquardt-Levenberg, Randomized Nelder-Mead, Random Shooting Method, and Generalized Simulated Annealing methods are the ones used for finding system parameters. The example results are shown in Figure 3.

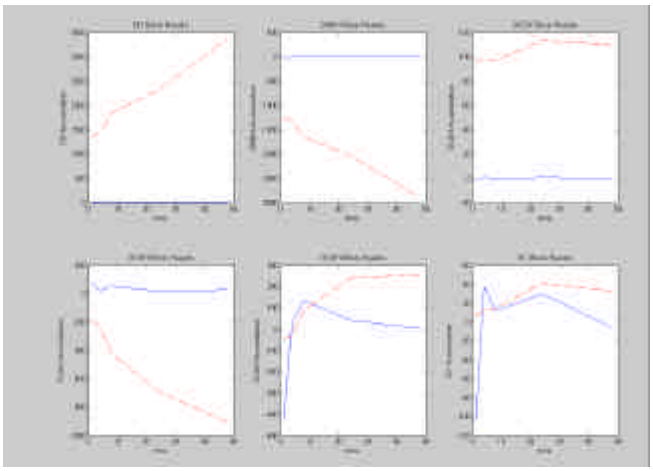


Figure 1 Linear Regression

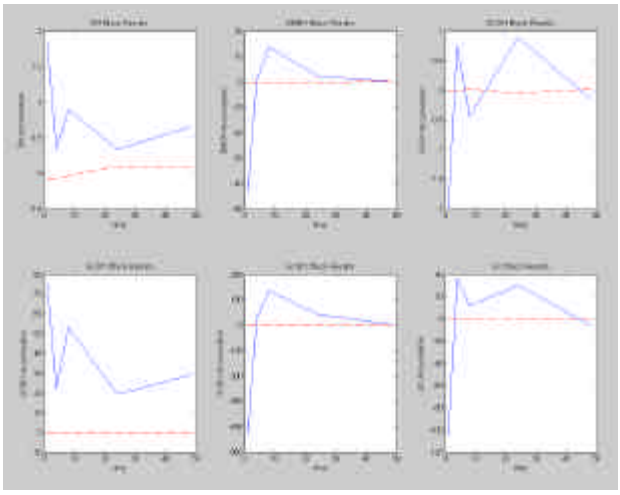


Figure 2 Comparison System Results using Marquardt-Levenberg

5. CONCLUSIONS AND FUTURE WORK

This system was initially built with a series of nonlinear forward flux equations as the mathematical model governing each reaction. Then it expands the biological relevance and accuracy of the predictive equations by including well-documented factors such as enzyme dependence and the reversible nature of reactions. Ultimately, the goal is to extend the integrated experimental and modeling methodologies illustrated in sphingolipid metabolism study to other complex biological process studies such as signal transduction or gene regulation. Another feature of our research is that the 3-D information representation enables the users to orchestrate the simulated pathway in real time.

REFERENCES

1. Merrill, A.H., *De novo sphingolipid biosynthesis: A necessary, but dangerous, pathway.* Journal of Biological Chemistry, 2002. **277**(29): p. 25843-25846.
2. Gibbs, J.B., *Mechanism-based target identification and drug discovery in cancer research.* Science, 2000. **287**(5460): p. 1969-1973.
3. Kitano, H., *Systems biology: A brief overview.* Science, 2002. **295**(5560): p. 1662-1664.
4. Bailey, J.E., *Lessons from metabolic engineering for functional genomics and drug discovery.* Nature Biotechnology, 1999. **17**(7): p. 616-618.
5. Cascante, M., et al., *Metabolic control analysis in drug discovery and disease.* Nature Biotechnology, 2002. **20**(3): p. 243-249.
6. Sander, C., *Genomic medicine and the future of health care.* Science, 2000. **287**(5460): p. 1977-1978.
7. Noble, D., *Modeling the heart - from genes to cells to the whole organ.* Science, 2002. **295**(5560): p. 1678-1682.
8. Kitano, H., *Computational systems biology.* Nature, 2002. **420**(6912): p. 206-210.
9. D. M. Bates and D. G. Watts, *Nonlinear regression analysis and its applications*, Wiley, New York, 1988.
10. J. H. Kalivas, *Adaption of simulated annealing to chemical optimization problems*, Elsevier, Amsterdam ; New York, 1995.
11. R. H. J. M. Otten and L. P. P. P. v. Ginneken, *The annealing algorithm*, Kluwer Academic Publishers, Boston, 1989.
12. J. F. Rusling and T. F. Kumosinski, *Nonlinear computer modeling of chemical and biochemical data*, Academic Press, San Diego, 1996.