CHALLENGES OF COMPUTER-AIDED DESIGN OF BIOPROCESSES: OPPORTUNITIES PRESENTED BY SYSTEMS BIOLOGY

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Abstract

As the scope of commercial manufacturing of biologic products has expanded recently both in terms of number of products and scale of operation, the need for computer-aided process design has likewise become greater. It is no longer unusual for a protein therapeutic to be manufactured at multiple sites at scales and productivities that greatly exceed what was current practice just a few years ago. Thus, the need for better process design has greatly increased yet the intrinsic complexity of biological processes complicates the application of computer-aided approaches. Taking a lead from recent developments in discovery research, a systems biology approach may serve these efforts better. Systems biology investigates the behavior and relationships of all the elements in a particular biological system while it is functioning. Furthermore, it seeks to predict the quantitative behavior of an in vivo biological process under a realistic range of conditions. The potential for using a systems biology approach to process design will be reviewed as well as a discussion of specific applications.

Keywords: FOCAPD 2004, Systems Biology, Bioprocess modeling.

Introduction: What is Systems Biology?

Systems Biology has recently emerged as a field of study that promises the potential for great advances in the overall understanding of biological systems. Perhaps the best description of Systems Biology comes from Leroy Hood in a recent interview (Hood and Carney, 2003):

> "*Systems biology is the ability to look at all of the elements in a biological system – by elements, I mean genes, messenger RNA, proteins, protein interactions and so forth – and to measure their relationships to one another as the system functions in response to biological or genetic perturbations. Then one can attempt to model the behaviour after integrating the different levels of information, either graphically or mathematically, so that,ultimately, you will be able to describe the behaviour of the system given any kind of perturbation. ... What distinguishes systems biology from the more classical biology of the past 35 years or so, which looked at genes and proteins one at a time, is the attempt to look at all, or at least most, of the elements and their interrelationships.*"

Systems biology, as a new discipline, draws not only from the recent historical developments in the fields of genomics and proteomics which Leroy Hood had pioneered but also parallels advances in the field of computer systems (Kitano, 2002). This interaction has its roots in the work of Norbert Weiner at MIT over 50 years ago. Weiner (1948) worked to create a vision for cybernetics: systems-level analysis and mathematical modeling of control and communication in animals and machines. Likewise, systems biology represents a combination of classical, hypothesis-driven science with the data-driven methods that have evolved with faster computation speeds (Kell and Oliver, 2003).

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Roots of Systems Biology in Chemical and Biochemical Engineering

One should also consider the rich history of cell modeling within the discipline of Chemical and Biochemical Engineering as another part of the rootstock of systems biology. As Doug Lauffenburger (2003a) said in a recent book review in *Cell*: *"Lately, the words "computation" and "cell biology" have begun to appear noticeably together in public, after a decade or more of discreet liaison*." Of course, the decade of *discreet liaison* in the area of cell biology was preceded by several decades of cell modeling that began with procaryotic systems. At about the same time Weiner was publishing his work on cybernetics, the foundations of structured cell models were being established by Monod (1949, 1950) in his work on growth rate models; followed by the work of Gaden (1959) on fermentation process kinetics and Luedeking and Piret (1959) on models for microbial product formation. Indeed, the current paradigms of systems biology can also be considered in the context of the historical lineage of modelers (whole cells, metabolic pathways, and signaling networks) within chemical and biochemical engineering that followed from then until the present day:

- A. Fredrickson (1976)
- J. Bailey (Lee and Bailey, 1984),
- M. Shuler (Domach *et al*, 1984),
- G. Stephanopoulos (Vallino and Stephanopoulos, 1993),
- D. Lauffenburger (Asthagiri *et al*, 1999),
- K. Lee (Lee and Lee, 2003),
- J. Liao (Bulter *et al*, 2003)
- B. Palsson (Covert *et al*, 2003)
- F. Srienc (Carlson and Srienc, 2004)

These works have helped establish a strong tradition in the modeling and quantification of cell growth, metabolism, and dynamics. In fact, an extensive amount of work is currently underway in trying to establish the best cellular models (Bley and Muller, 2002; Wolkenhauer, 2002; Nielsen and Olsson, 2002; Csete and Doyle, 2002; Levchenko, 2003; Patil *et al*, 2004).

Systems Biology and Drug Discovery

Great hope is being placed on the potential for systems biology to help accelerate and improve the efficiency of the drug discovery process (Huels *et al*, 2002; Davidov *et al*, 2003). Drug discovery, which has only existed as an industrialized institutional activity for about 100 years, has undergone a tremendous acceleration in recent years through better understanding of metabolic control pathways and by using high-throughput screening (HTS) methods to identify therapeutics against specific targets (Drews, 2000). For example, gene expression monitoring has led to a better understanding of the classification of cancers (Golub *et al*, 1999). By extension, certain classes of targets (*e.g.*, protein kinases) have emerged as prime targets for cancer therapies (Fabbro *et al*, 2002). However, success is not always guaranteed for those following a single target, especially when treating cancer which often develops in an individual as an accumulation of multiple unique insults to normal cellular control motifs. In this case, broader transcriptional profiling may yield better results (Aburatani, 2002). Also, understanding of historical model systems used for target identification and validation can be improved by the application of better tools (Sharom *et al*, 2004; Carroll *et al*, 2003). By extension, these approaches will lead the way to a fuller systems biology analysis in terms of understanding the best therapeutic route. As Cockett *et al* (2000) have pointed out:

> "*Because of the high attrition rates inherent in the development of novel medicines, >60 new targets entering discovery and 20 drug candidates entering clinical trials are needed every year to produce three novel drugs annually. The goal of applied genomics within pharmaceutical research and development is to help sustain the overall output by providing novel high-quality targets as well as by reducing the percentages of preclinical and clinical failures."*

Adding the feedback loop of pharmacogenomics to the overall process of drug discovery and development should improve the chances for ultimate success in the clinic. That is, by better understanding the underlying mechanisms that cause stratification of patient response, not only can those patients more likely to benefit from a given therapy be selected up front, new unmet medical needs can also be identified more precisely. Thus, application of systems biology to the overall process of drug discovery should yield a higher output of successful therapeutics.

Co-Evolution of Drug Discovery and Biochemical Engineering Case Study: Baker's Yeast Production and Tumor Biology Research

It is interesting to note the parallels that exist in the development of chemical and biochemical engineering and drug discovery. The parallel evolution of these fields have been enriched by a number of interconnections. The most recent example of cross-talk between them can be viewed as systems biology, yet a number of precedents can be identified. These multiple interactions can be highlighted by considering as a case study the connections between tumor biology research and the commercial production of baker's yeast.

Nearly every article over the past 35 years on the modern production of baker's yeast refers to one physiological touchstone: the central importance of the Crabtree effect on glucose metabolism and the need to control it in order to maximize production. Yet essentially no one refers to the fact that Crabtree (1928) was studying the glucose metabolism of tumors when he published his results. In fact, it took almost 40 years for DeDeken (1966) to establish that a parallel metabolic pathway existed in yeast. The connection between this "overflow" metabolism and the establishment of efficient means for producing baker's yeast took another decade. That is, as an understanding developed between the glucose concentration in the medium and the production of ethanol (which reduced the yield of biomass), more efficient feeding schemes evolved. This approach benefited from advances in computing technology and was further improved by the use of a computer-controlled feeding scheme based on a mass balance model (Wang *et al*, 1977). Although more than 25 years have passed since the publication of Wang's work, optimization of Baker's yeast production is still being investigated using: (1) an engineering scale-down system (George *et al*, 1999), metabolic flux models (Forster *et al*, 2002; Thierie, 2004), and basic genetics (Rincon *et al*, 2001). We are making progress but don't appear to have established the ultimate solution to an optimal large scale production process for Baker's yeast.

DeDeken's work in the 1960s occured during a period that also included the beginnings of more advanced genetic studies on this organism. It was around this time that mapping of the *Saccharomyces* genome was begun (Hawthorne and Mortimer, 1960). Again, with advances in technology, the complete sequence of the *Saccharomyces* genome was completed in 1996 (Goffeau *et al*, 1996), although characterization of the ~6000 genes as to isolated function using traditional methods is projected for around 2010 (Johnston, 2000). However, on a parallel path, some investigators are developing complex mathematical models of the yeast cell. Metabolic network analyses are common (Sainz, 2003; Ostergaard, 2001). This approach can be extended to better control the feed to fermentations (Chang, 2000). However, an even more exciting approach is the modeling of signal transduction pathways combined with expression data input (Lin *et al*, 2003). Such a model should give a much better prediction of the dynamic response of cells under varying conditions and could be considered part of the foundation of the systems biology of yeast.

Going back to where we started - Crabtree's work certainly had an impact on tumor biology research in his day (Dickens, 1930). This theme continued through the 1960s (Letnansky, 1968) when DeDeken was working on yeast and the 1970s (Sussman *et al*, 1980) when Wang was working on computer control of fermentations and into the present day (Burd *et al*, 2001; Rodriguez-Enriquez, 2001). However, these studies have become increasingly sophisticated as analytical and computational tools have advanced. In fact, some of the recent work has begun to organize the information about the altered metabolism of tumors into more sophisticated multi-factor models that could be considered part of systems biology (Dang and Semenza, 1999; and Gatenby and Vincent, 2003).

Thus, the path forward from Crabtree in 1929 has led to improved understanding of both tumor and yeast metabolism unified by the sort of integrated models that have become part of systems biology. This case study is also typical in that it illuminates the many intertwined paths between pure discovery and engineering science that can contribute to advances in both fields.

Development of Fed-Batch Fermentations and the Status of Process Modeling and Control

Moving on from Baker's yeast, the use of fed-batch fermentations has become increasingly commonplace in the biotechnology industry. Following the traditional paradigm, this type of process has been used for the production of primary metabolites, biomass, secondary metabolites, protein expressed in *E. coli* or yeast and finally protein expressed in mammalian cells. It is commonly used in cost-sensitive processes because it can deliver the highest product concentration to the purification process and thus minimize the impact of fermentation costs. Over time a number of fed-batch control strategies have developed (Lee *et al*, 1999):

- pre-determined or feed-forward strategies
- simple, indirect feedback control
- feed according to measured nutrient uptake or demand
- feed according to an inferred or calculated demand (or growth rate) based on single measurements
- feed according to a model of nutrient demand or cell growth based on multiple measurements
- feed according to a "fuzzy" model or neural network representation of a process model

Currently, the most attention, at least in an industrial situation, is placed on the optimization of a simple feedback control strategy using a model-based strategy (Lubbert and Jorgensen, 2001). Tuning and optimization of fed-batch process control remains an area of current interest (Muthuswamy and Srinivasan, 2003; James *et al*, 2002; Akesson *et al*, 2001) and the quality of the underlying model is always of utmost importance.

Current Unmet Needs in Biotechnology

The current landscape of commercial biotechnology products has changed dramatically over the last 20 to 25 years. Therapeutics have evolved from low-volume, high value-added specialty products that were typically produced at a single manufacturing site into high-volume commodities produced at multiple sites for which production cost issues have become increasingly critical. Complicating the current status is that many of these high-volume products, such as monoclonal antibodies (mAbs) or related proteins, are made by mammalian cell culture. For example, Genentech has seven commercial products produced by mammalian cell culture (four mAbs and three others). Supplying the market has required hundreds of batches at the 12,000L scale from four sites worldwide. Another good example is Enbrel: Amgen produces this billion-dollar product using a network of five bulk manufacturing sites (operating three different processes) and three drug product sites. The multiple challenges here are as follows:

1. **Highly expanded scope of manufacturing**
   - mammalian cell culture has been historically difficult to scale (or at least more difficult than bacterial and yeast fermentations)
   - as identified above, supply chain requirements for these products often requires multiple sites for both bulk drug substance and drug product, often including third party sites
   - dosing has increased as products have moved from being catalytic in nature (G-CSF, interferons) to stoichiometric or blocking (Enbrel, Remicade)

2. **Exposure to a greater number patients**
   - the target population for many current therapies has shifted from acute treatment of life-threatening diseases in a small population to chronic treatment or disease management in a much larger, often healthier population
   - thus, more people (and typically more healthier people) are exposed over a longer time to a given therapeutic

3. **Products more difficult to characterize**
   - the products made from mammalian cell culture are typically more complex and require more complicated characterization
   - part of the complexity results from post-translational modifications (e.g. glycosylation, often at multiple locations on the protein) that make full characterization even more complicated

Thus the demands for producing a comparable product are greater than ever. The FDA has certainly recognized the issues around comparability (Mire-Sluis, 2003) and has served as arbitrator for efforts in industry to develop better methods. Also, good process control has been successful in yielding comparable products. However, as more products are submitted for approval, the pressure will become greater to maintain consistent quality while delivering both the quantity demanded and at the price required. This pressure is analogous to what drove continued improvements in the process control of Baker's yeast at ever-increasing scales but is many times more complex.

Impact of Systems Biology on Process Development

How will the Biotechnology industry resolve the issues outlined above? Certainly the drive for better process control from a quality perspective will have an impact. This can be accomplished through better identification of critical operating parameters (Buck *et al*, 2002) or more sophisticated approaches to process monitoring (Zhang and Lennox, 2004; Lennox *et al*, 2001). However, the impact of tightening the general control strategy for a bioprocess will be limited by the degree of "inherent" variability in the process.

Control of biological variability can only be approached through better understanding of the underlying mechanisms controlling product formation, post-translational modification, and degradation. In some cases, it may be necessary to start with a basic re-engineering of production cell lines. However, for reasonably robust processes, better understanding of the interaction of cell behavior and process control may be all that is required. Certainly a great need exists for better models of mammalian cell processes from a single cell perspective. As indicated above, we are just beginning to understand simpler organisms such as *E. coli* or yeast, so mammalian cells represent a much greater challenge. Engineering science may be able to borrow from basic research; many of the models of systems biology investigations (*e.g.*, as survival signaling) may be directly applicable to cell culture. However, other areas, such as the understanding of the intrinsic biology of protein expression (and particularly post-translational modifications) at a systems level, may be more specific to this field. A recent review by Komives and Parker (2003) suggests that using more sophisticated models (or combinations of models) for bioprocess can lead to improved performance. They claim the quality of the output from a model increases with the complexity of that model or estimator, and that coupling multiple models also improved performance. The direction they suggest is essentially that of a systems biology approach to protein production.

Regarding the large scale performance of bioreactors, understanding the biology at a systems level may be necessary for better control but it is probably not completely sufficient. Large scale bioreactors present a complex hydrodynamic environment that affects mixing, hydrodynamic shear, and (air/liquid) interfacial shear. The need certainly exists to understand these scale-up effects and to be able to integrate them with physical and metabolic effects. Again, it is only recently that greater experience with large scale fermentations and better tools for process and biological monitoring have led to more detailed analysis of this issue (Enfors *et al*, 2001). More sophisticated hydrodynamic models have been combined with a better understanding of stress-related pathways within the cell, combined with methods for monitoring them, to allow new insight into the scale-up process. This has improved the quality of scale-down simulations as well (Bylund, 1999). The ultimate goal would be the integration of fluid dynamic modeling with an understanding of the biology at a systems level. Some investigators have begun to approach this for microbial systems (Vrabel, 2001) but the need to extend this for mammalian cell culture is clear.

Finally, the impact of systems biology can be felt in the way scientists and engineers go about the process of process development. That is, the way problems are structured in systems biology could affect the way people think about problems and even how they organize process development efforts. For example, in a recent presentation, Lauffenburger (2003b) presented the model below for the integrated way in which systems biology combines hypothesis-driven and data-driven inquiry.

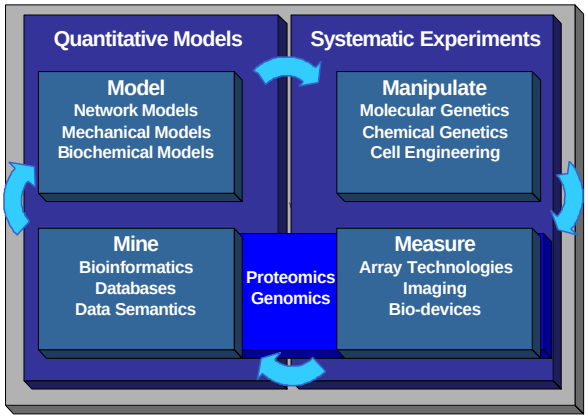

Figure 1: The "Four Ms" of Systems Biology. (Lauffenburger, 2003b)

This structured approach to problems could also be considered as a part of developing a model for integrating process research and development with manufacturing operations.

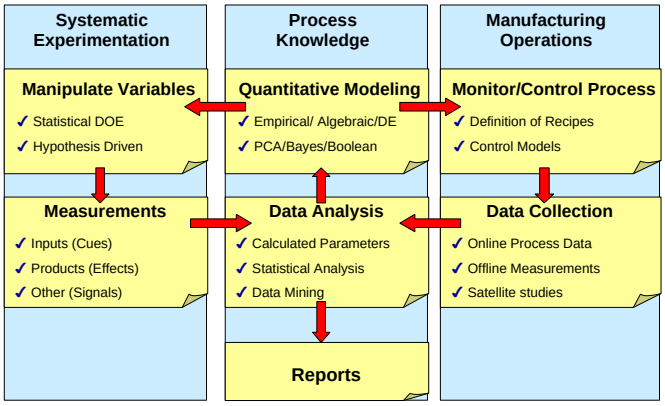

Figure 2. A paradigm for process research and development.

References

Aburatani, H. (2002) Understanding cancer through gene expression profiling. *International Congress Series* 1246, 261– 270.

Akesson, M., P. Hagander, and J. P. Axelsson (2001). Probing control of fed-batch cultivations: analysis and tuning. *Control Engineering Practice* 9, 709-723.

Asthagiri, A. R., C. M. Nelson, A. F. Horwitz, and D. A. Lauffenburger (1999). Quantitative Relationship among Integrin-Ligand Binding, Adhesion, and Signaling via Focal Adhesion Kinase and Extracellular Signal-regulated Kinase. *Journal of Biological Chemistry* 274, 27119–27127.

Bachinger, T., U. Riese, Rolf Eriksson, and C.-F. Mandenius (2000). Monitoring cellular state transitions in a production-scale CHO-cell process using an electronic nose. *Journal of Biotechnology* 76, 61–71.

Barnes, L. M., C. M. Bentley, and A. J. Dickson (2004). Molecular Definition of Predictive Indicators of Stable Protein Expression in Recombinant NS0 Myeloma Cells. *Biotechnology & Bioengineering*, 85, 115-121.

Bley, T. and S. Muller (2002). How Should Microbial Life be Quantified to Optimise Bioprocesses? *Acta Biotechnol*. 22, 401-409.

Buck, K. K. S., V. Subramanian, and D. E. Block (2002). Identification of Critical Batch Operating Parameters in Fed-Batch Recombinant E. coli Fermentations Using Decision Tree Analysis. *Biotechnol. Prog*. 18, 1366-1376.

Bulter, T., J. R. Bernstein, and J. C. Liao (2003). A Perspective of Metabolic Engineering Strategies: Moving up the Systems Hierarchy. *Biotechnology & Bioengineering* 84, 815-821.

Bylund, F., F. Guillard, S.-O. Enfors, C. Tragardh, G. Larsson (1999). Scale down of recombinant protein production: a comparative study of scaling performance. *Bioprocess Engineering* 20, 377-389.

Carlson, M. (1999). Glucose Repression in Yeast. *Current Opinion in Microbiology* 2, 202-207.

Carlson, R. and F. Srienc (2004). Fundamental Escherichia coli Biochemical Pathways for Biomass and Energy Production: Identification of Reactions. *Biotechnology & Bioengineering* 85, 1-19.

Carroll, P. M., B. Dougherty, P. Ross-Macdonald, K. Browman, and K. FitzGerald (2003). Model systems in drug discovery: chemical genetics meets genomics. *Pharmacology & Therapeutics* 99, 183– 220.

Chang, D. (2003). The Snowball Effect in Fed-Batch Bioreactions. *Biotechnol. Prog*. 19, 1064-1070.

Chapman, S. and A. R. Asthagiri (2004). Resistance to Signal Activation Governs Design Features of the MAP Kinase Signaling Module. *Biotechnology & Bioengineering* 99, 1-12.

Cockett, M., N. Dracopoli and E. Sigal (2000). Applied genomics: integration of the technology within pharmaceutical research and development. *Current Opinion in Biotechnology* 11, 602–609.

Covert, M. W., I. Famili, and B. O. Palsson (2003). Identifying Constraints that Govern Cell Behavior, A Key to Converting Conceptual to Computational Models in Biology? *Biotechnology & Bioengineering*, 84, 763-772.

Crabtree, H.G. (1929). Observations on the carbohydrate metabolism of tumours. *Biochem. J.* 23, 536-545.

Csete, M. E. and J. C. Doyle (2003). Reverse Engineering of Biological Complexity. *Science* 295, 1664-1669.

Dang, C. V. and G. L. Semenza (1999). Oncogenic alterations of metabolism. *Trends Biochem. Sci.* 24, 68-72.

Davidov, E. J., J. M. Holland, Edward W. Marple and Stephen Naylor (2003). Advancing drug discovery through systems biology. *Drug Discovery Today* 8, 175-183.

DeDeken, R. H. (1966). The Crabtree Effect: A Regulatory System in Yeast. *J. Gen. Microbiol*. 44, 149-156.

Dickens, F. and F. Simer (1930). A Note on the Metabolism of Tumours *The Lancet* July 5, 10-12.

Drews, J. (2000). Drug Discovery: A Historical Perspective. *Science* 287, 1960-1964.

Dickens, F., M. A. Camb, and F. Simer (1930). A Note on the Metabolism of Tumours. *The Lancet* July 5, 10-12.

Domach, M. M., S. K. Leung, R. E. Cahn, G. G. Cocks, and M. L. Shuler (1984). Computer Model for Glucose-Limited Growth of a Single Cell of *Escherichia coli* B/r-A. *Biotechnology & Bioengineering* 26, 203-216.

Enfors, S.-O., M. Jahic, A. Rozkov, B. Xu, M. Hecker, B. Jurgen, E. Kruger, T. Schweder, G. Hamer, D. O'Beirne, N. Noisommit-Rizzi, M. Reuss, L. Boone, C. Hewitt, C. McFarlane, A. Nienow, T. Kovacs, C. Tragardh, L. Fuchs, J. Revstedt, P. C. Friberg, B. Hjertager, G. Blomsten, H. Skogman, S. Hjort, F. Hoeks, H.-Y. Linm, P. Neubauer, R. van der Lans, K. Luyben, P. Vrabel, A,. Manelius (2001). Physiological responses to mixing in large scale bioreactors. *Journal of Biotechnology* 85 175–185.

Fabbro, D., S. Ruetz, E. Buchdunger, S. W. Cowan-Jacob, G. Fendrich, J. Liebetanz, J. Mestan, T. O'Reilly, P. Traxler, B. Chaudhuri, H. Fretz, J. Zimmermann, T. Meyer, G. Caravatti, P. Furet, and P. W. Manley (2002). Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. *Pharmacology & Therapeutics* 93, 79– 98.

Ferreira, L. S., M. B. De Souza, Jr, J. O. Trierweiler, O. Broxtermann, R. O. M. Folly, and B. Hitzmann (2003). Aspects concerning the use of biosensors for process control: experimental and simulation investigations. *Computers and Chemical Engineering* 27, 1165-1173.

Forster, J., A. K. Gombert, and J. Nielsen (2002). A Functional Genomics Approach using Metabolomics and *in silico* Pathway Analysis. *Biotechnology & Bioengineering* 79, 703-712.

Fox, S. R., U. A. Patel, M. G. S. Yap, and D. I. C. Wang (2004). Maximizing Interferon–gamma Production by Chinese Hamster Ovary Cells Through Temperature Shift Optimization: Experimental and Modeling. *Biotechnology & Bioengineering* 85, 177-184.

Frederickson, A. G. (1976). Formulation of Structured growth Models. *Biotechnology & Bioengineering* 18, 1481-1486.

Fussenegger M, and J. E. Bailey (1999). Control of mammalian cell proliferation as an important strategy in cell culture technology, cancer therapy and tissue engineering. In: Al-Rubeai, Hauser, Jenkins, Betenbaugh, McDonald, editors. *Cell engineering I.* Norwell, MA: Kluwer Academic. p 186-219.

Gaden, E. L. (1959) Fermentation Process Kinetics. *Journal of Biochemical and Microbiological Technology and Engineering* 1, 413–429.

Gancedo, J. M. (1998). Yeast Carbon Catabolite Repression. *Microbiology & Molecular Biology Reviews*. 62, 334–361.

Gatenby, R. A. and T. L. Vincent (2003). An Evolutionary Model Of Carcinogenesis. *Cancer Research* 63, 6212-6220.

George, S., G. Larsson, K. Olsson, S.-O. Enfors (1998). Comparison of the Baker's yeast process performance in laboratory and production scale. *Bioprocess Engineering* 18, 135-142.

Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, E. J. Louis, H. W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin, S. G. Oliver (1996). Life with 6000 Genes. *Science* 274, 563-567.

Golub, T. R., D. K. Slonim, P. Tamayo, C. Huard, M. Gaasenbeek, J. P. Mesirov, H. Coller, M. L. Loh, J. R. Downing, M. A. Caligiuri, C. D. BloomÞeld, E. S. Lander (1999). Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring. *Science* 531-537.

Gosalvez, M., S. Garcia-Suarez and L Lopez-Alarcon (1978). Metabolic control of glycolysis in normal and tumor permeabilized cells. *Cancer Research* 38, 142-148.

Harms, P., Y. Kostov and G. Rao (2002). Bioprocess monitoring. *Current Opinion in Biotechnology* 13, 124–127.

Hawthorne, D. and R. K. Mortimer (1960). Chromosome mapping in *Saccharomyces*: centromere-linked genes. *Genetics* 45, 1085-1110.

Hood, L. and S. Carney (2003). Leroy Hood expounds the principles, practice, and future of systems biology. *Drug Discovery Today* 8, 436-438.

Huels, C., S. Muellner, H. E. Meyer, and D. J. Cahill (2002). The impact of protein biochips and microarrays on the drug development process. *Drug Discovery Today* 7, S119-S124.

Ingalls, B. P. and H. M. Sauro (2003). Sensitivity analysis of stoichiometric networks:an extension of metabolic control analysis to non-steady state trajectories. *Journal of Theoretical Biology* 222, 23 –36.

James, S., R. Legge, R. Budman (2002). Comparative study of black-box and hybrid estimation methods in fed-batch fermentation. *Journal of Process Control* 12, 113-121.

Johnston, M. (2000). The yeast genome: on the road to the Golden Age. *Current Opinion in Genetics & Development* 10, 617–623.

Jones, K. D. and D. S. Kompala (1999). Cybernetic model of the growth dynamics of Saccharomyces cerevisiae in batch and continuous cultures. *Journal of Biotechnology* 71, 105–131.

Kell, D. B. and S. G. Oliver (2004). Here is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era. *BioEssays* 26, 99–105.

Kitano, H. (2002). Systems Biology: A Brief Overview. *Science* 295, 1662-1664.

Klapa, M. and J. Quackenbush (2003). The Quest for the Mechanisms of Life. *Biotechnology & Bioengineering*, 84, 739-742.

Komives, C. and R. S. Parker (2003). Bioreactor state estimation and control. *Current Opinion in Biotechnology* 14, 468–474.

Lauffenburger, D. A. (2003a). Book Review: Modeling Cells Adds Up. *Cell* 113, 7-8.

Lauffenburger, D. A. (2003b). Presentation: Computational and Systems Biology at MIT. Dallas.

Lee, S. B. and J. E. Bailey (1984). Analysis of Growth Rate Effects on Productivity of *Escherichia coli* Populations Using Molecular Mechanism Models. *Biotechnology and Bioengineering* 26, 66-73.

Lee, P. S. and K. H. Lee (2003). Escherichia coli—A Model System That Benefits From and Contributes to the Evolution of Proteomics. *Biotechnology & Bioengineering* 84, 801-814.

Lee, J., S. Y. Lee, S. Park, and A. P. J. Middelberg (1999). Control of fed-batch fermentations. *Biotechnology Advances* 17, 29–48.

Lennox, B., G. A. Montague, H. G. Hiden, G. Kornfeld, and P. R. Goulding (2001). Process Monitoring of an Industrial Fed-Batch Fermentation. *Biotechnology & Bioengineering* 74, 125-135.

Letnansky, K. (1968). Strain Variations in the Utilization of Hexoses by Ehrlich Ascites Tumor Cells. *Biochim.et Biophys. Acta* 165, 364-373.

Levchenko, L. (2003). Dynamical and Integrative Cell Signaling: Challenges for the New Biology. *Biotechnology & Bioengineering* 84, 773-782.

Lin, X., C. A. Floudas, Y. Wang, and J. R. Broach (2003). Theoretical and Computational Studies of the Glucose Signaling Pathways in Yeast Using Global Gene Expression Data. *Biotechnology & Bioengineering* 84, 864-886.

Lubbert, A. and S. B. Jørgensen (2001). Bioreactor performance: a more scientific approach for practice. *Journal of Biotechnology* 85, 187–212.

Luedeking, R. and E. L. Piret (1959). A Kinetic Study of the Lactic Acid Fermentation. Batch Process at Controlled pH. *Journal of Biochemical and Microbiological Technology and Engineering* 1, 393–412.

Mangan, S., A. Zaslaver, and U. Alon (2003). The Coherent Feedforward Loop Serves as a Sign-sensitive Delay Element in Transcription Networks. *J. Mol. Biol*. 334, 197–204.

Mire-Sluis, A. R. (2003). Current Perspectives on the use of Analytical Data to show Comparability. *Biologics 2003* Presentation.

Monod, J. (1949) *Ann. Rev. Microb*. 3, 371.

Monod, J. (1950) *Ann. Inst Pasteur* 79, 390.

Muthuswamy, K. and R. Srinivasan (2003). Phase-based supervisory control for fermentation process development. *Journal of Process Control* 13, 367 – 382.

Nielsen, J. and L. Olsson (2002). An expanded role for microbial physiology in metabolic engineering and functional genomics: moving towards systems biology. *FEMS Yeast Research* 2, 175-181.

Ostergaard, S., L. Olsson, J. Nielsen (2001) In Vivo Dynamics of Galactose Metabolism in *Saccharomyces cerevisiae*: Metabolic Fluxes and Metabolite Levels. *Biotechnology and Bioengineering* 73, 412-425.

Patil, K. R., M. Akesson, and J. Nielsen (2004). Use of genome-scale microbial models for metabolic engineering. *Current Opinion in Biotechnology* 15, 1–6.

Rincon, A. M., A. C. Codon, F. Castrejon, and T. A. Benitez (2001). Improved Properties of Baker's Yeast Mutants Resistant to 2-Deoxy-D-Glucose. *Applied & Environmental Microbiology* 67, 4279–4285.

Rivenzon-Segal, D., R. Margalit, and H. Degani (2002). Glycolysis as a metabolic marker in orthotopic breast cancer, monitored by *in vivo* 13C MRS. *Am J Physiol Endocrinol Metab* 283: E623-E630.

Rodriguez-Enriquez, S., O. Juarez, J. S. Rodriguez-Zavala and R. Moreno-Sanchez (2001). Multisite control of the Crabtree effect in ascites hepatoma cells. *Eur. J. Biochem*. 268, 2512-2519.

Rolland, F., J. Winderickx, and J. M. Thevelein (2001). Glucose-sensing mechanisms in eukaryotic cells. *TRENDS in Biochemical Sciences* 26, 310-317.

Sainz, J., F. Pizarro, J. R. Perez-Correa, E. Agosin (2003). Modeling of Yeast Metabolism and Process Dynamics in Batch Fermentation. *Biotechnology and Bioengineering* 81, 818-828.

Schugerl, K. (2001). Progress in monitoring, modeling and control of bioprocesses during the last 20 years. *Journal of Biotechnology* 85, 149–173.

Sharom, J. R., D. S. Bellows, and M. Tyers (2004). From large networks to small molecules. *Current Opinion in Chemical Biology* 8, 1–10.

Sussman, I., M. Ereciska, and D. F. Wilson (1980). Regulation of Cellular Energy Metabolism: The Crabtree Effect. *Biochimica et Biophysica Acta*, 591, 209-223.

Takors, R., M. Gerigck, H. Paschold, and C. Wandrey (2001). Principal-component analysis of Microbial L-Phenylalanine Production. *Bioprocess and Biosystems Engineering* 24, 93-99.

Thierie, J. (2004). Modeling threshold phenomena,metabolic pathways switches and signals in chemostat-cultivated cells:the Crabtree effect in Saccharomyces cerevisiae. *Journal of Theoretical Biology* (in press).

Thiry, M. and D. Cingolani (2002). Optimizing scale-up fermentation processes. *Trends in Biotechnology* 20, 103-105.

Tsoka, S. and C. A. Ouzounis (2003).Metabolic Database Systems for the Analysis of Genome-Wide Function. *Biotechnology & Bioengineering* 84, 750-755.

van Someren, E. P., L. F. A. Wessels, E. Backer, and M. J. T. Reinders (2003). Multi-criterion optimization for genetic network modeling. *Signal Processing* 83, 763 – 775.

Vallino, J. J. and G. Stephanopoulos (1993). Metabolic Flux Distributions in *Corynebacterium glutamicum* during Growth and Lysine Overproduction. *Biotechnology & Bioengineering* 41, 633-646.

Varner, J. D. (2000). Large-Scale Prediction of Phenotype: Concept. *Biotechnology & Bioengineering* 69, 664-678.

Vrabel, P., R. G.J.M. van der Lans, F. N. van der Schot, K. C. A. M. Luyben, B. Xu, and S. Enfors (2001). CMA: integration of fluid dynamics and microbial kinetics in modelling of large-scale fermentations. *Chemical Engineering Journal* 84, 463–474.

Weiner, N. (1948). *Cybernetics or Control and Communication in the Animal and Machine*, MIT Press.

Wolf, G., J. S. Almeida, C. Pinheiro, V. Correia, C. Rodrigues, M. A. M. Reis, and J. G. Crespo (2001). Two-Dimensional Fluorometry Coupled with Artificial Neural Networks: A Novel Method for On-Line Monitoring of Complex Biological Processes. *Biotechnology & Bioengineering* 72, 297-306.

Wolkenhauer , O. (2002). Mathematical modeling in the post-genome era: understanding genome expression and regulation - a system theoretic approach. *BioSystems* 65, 1–18.

Zhang, H. and B. Lennox (2004). Integrated condition monitoring and control of fed-batch fermentation processes. *Journal of Process Control* 14, 41 –50.