Call for papers

Oral presentations will be given by invited speakers and by contributing participants. Abstracts (about 200 words) for oral and poster presentations are welcomed. Authors will be notified about acceptance.

Deadlines

Abstracts: October 30, 2008

(200-300 words, e-mail: ***info@sysbiol.net***

Registration

Registration is free

For further information:

http://www.sysbiol.net/

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MINISTERIO DE CIENCIA E INNOVACIÓN

SYSBIOL2006

4th Meeting of the Spanish Systems Biology Network (REBS):

From genomes to *in silico* and back.

Organized under the auspices of Spanish Ministry of Science and Innovation

Second announcement

CALL FOR ORAL PRESENTATIONS

December 1st-2nd, 2008, Valencia, Spain

Scope

The major objective of this Meeting is to point out the importance of Systems Biology in describing a biological system. Biological systems consist of a large number of heterogeneous components interacting selectively with other components in the system. These components must be connected in a proper way, so that an entire system can be functional. Precise molecular models are required to represent and understand biological systems, opening a broad field of applications. Thus, the exchange of information and experience in research and close communication between international participants will help identify future needs and new aspects in the use of this new scientific field. An intense feedback between fundamentals and applications of bioprocess and biomedicine research will be stimulated.

Topics

- Fundamentals and tools of Systems Biology.
- Role and function of regulatory networks and signal transduction pathways.
- Systems Biology applications in bioprocesses.
- Systems Biology applications in biomedicine.

Organizing committee

Julio Banga	*IIM-CSIC (Spain)*
Manuel Cánovas	***University of Murcia (Spain).***
Marta Cascante	***University of Barcelona (Spain).***
S. F. Elena	***IBMCP-CSIC (Spain)***
N. Torres	***University of La Laguna (Spain).***

Scientific programme

The programme will comprise oral communications (15-30 min) and poster sessions. There will be no parallel sessions. Six plenary lectures are also programmed.

Plenary speakers

- **Walter van Gulick.** *University of Delft-DTU (Delft, Netherland)*
- **Vassily Hatzimanikatis.** *EPFL (Lausanne, Switzerland*) (To be confirmed).
- **Ian Booth**, *University of Aberdeen (Aberdeen, Scotland).*
- **Jörg Stelling**, *ETH Zürich (Zürich, Switzerland).*
- **Olaf Wolkenhauer,** *University of Rostock (Rostock, Germany)*
- Pedro Mendes, *University of Manchester (Manchester, UK)*

Location

The Conference will be held in the Sidi Saler Hotel (www.sidisaler.com), very close to the city of Valencia. The nearest airport is the International Airport of Valencia, located at 22 km from the Hotel. More info can be found on the web-site: www.sysbiol.net

IVth Meeting of the Spanish Systems Biology Network (REBS)

Hotel Sidi Saler, Valencia, December 1st-2nd, 2008

Sunday November 30th, 2008. Reception

Monday December 1st, 2008

9:00 – 9:15 Presentation: Santiago F. Elena & Manuel Cánovas

Session 1: Modeling and optimization.

Chairman: Néstor V. Torres

9:15 – 10:00 Invited speaker. Olaf Wolkenhauer, University of Rostock. "(Re)Constructing realities in Systems Biology: the Jacobian way".

10:00 – 10:20 Albert Sorribas, Universitat de Lleida. "A global optimization strategy for investigating operating design principles in metabolism: adaptive response of yeast to heat shock"

10:20 – 10:40 Francisco J. Planes. Universidad de Navarra. "Elementary flux modes and optimization".

10:40 – 11:00 Javier Buceta, Universitat de Barcelona. "Modeling protein dilution experiments: stochastic effects".

11:00 – 11:20 Julio R. Banga. IIM-CSIC. "Optimization and optimality in Systems Biology".

11:20 – 11:40 G. G. de Polavieja, IC-CSIC. "Suboptimality in Systems Biology".

11:40 – 12:00 Coffee break

Session 2: New analytical tools.

Chairman: Jesús Picó

12:00 – 12:45 Invited speaker. Vassily Hatzimanikatis, EPF Lausanne. "Computational metabolic engineering".

12:45 – 13:05 Jesús Picó. Universidad Politécnica de Valencia. "A possibilistic framework for metabolic flux analysis".

13:05 – 13:25 Joan Segura, CNB-CSIC. "aGEM: an integrative system for the analysis of spatial-temporal gene expression information".

13:25 – 13:45 Joaquin Dopazo. CIPF. "Casting genomic data into Systems Biology concepts".

13:45 – 14:05 Eduardo Pareja, ERA7. "Symmetric monoidal (bi)categories with feedback and biological networks".

14:05 – 15:30 Lunch

Session 3: Systems Biology of Microorganisms.

Chairman: Manuel Cánovas

15:30 – 16:15 Invited speaker. Ian Booth, University of Aberdeen. "Systems Biology: a physiological journey from El Greco to Matisse - the art of science".

16:15 - 16:35 Max Chavarría, CNB-CSIC. "The regulatory duties of the phosphotransferase system (PTS^{Ntr}): A metabolic flux analysis of *Pseudomonas putida*".

16:35 – 16:55 Eva Yus, CRG. "Systems Biology of *Mycoplasma*, a minimal organism".

16:55 – 17:15 José Ramos. Universidad de Córdoba. "Biochemical and proteomic approaches to the study of potassium homeostasis in *Saccharomyces cerevisiase*".

17:15 – 17:35 Joaquín Ariño, Universitat Autònoma de Barcelona. "Modeling cation homeostasis in the yeast *Saccharomyces cerevisiae*: A gene discovering and transcriptomic profiling approach".

17:35 – 17:55 Pau Ferrer, Universitat Autònoma de Barcelona. "A multi-level approach to the study of heterologus protein production in *Pichia pastoris* under different oxygen conditions".

17:55 – 18:15 Coffee break

Session 4: Metabolic Engineering and Synthetic Biology.

Chairman: Andrés Moya

18:15 – 19:00 Invited speaker. Jörg Stelling, ETH Zürich. "Computational design of synthetic circuits".

19:00 – 19:20 Andreea Munteanu, Universitat Pompeu Fabra. "Neutrality and robustness in evo-devo".

19:20 – 19:40 Luis Delaye, Universitat de València. "Engineering a minimum photoautotrophic cell: the case of *Synechococcus elongatus* PCC7492".

19:40 – 20:00 Rui Alves, Universitat de Lleida. "Bacterial two component systems".

20:00 – 20:45 Invited speaker. Pedro Mendes, University of Manchester. "A new strategy for assessing global sensitivity in biochemical networks".

20:45 – 21:15 Poster session

21:15 – 22:30 Dinner

Tuesday December 2nd, 2008

Session 5: Applications: Biomedicine.

Chairman: Joaquin Dopazo

9:00 – 9:45 Invited speaker. Walter van Gulik. Delft University of Technology. "Methods in quantitative microbial metabolomics: how to get the right numbers?".

9:45 – 10:05 Marta Cascante, Universitat de Barcelona. "A Systems Biology approach to multifactorial diseases".

10:05 – 10:25 Adrián L. García de Lomana. Universitat Pompeu Fabra. "Global connectivity and activity distributions in cellular networks".

10:25 – 10:45 Juan C. Argüelles, Universidad de Murcia. "Research of trehalose in pathogenic yeast as a useful model of infectivity for plants and mammals".

10:45 – 11:05 Amparo Querol, IATA-CSIC. "Comparative transcriptomic between virulent and avirulent *Saccharomyces cerevisiae* isolated from human blood".

11:05 – 11:25 Santiago F. Elena, IBMCP-CSIC. "Virus adaptation by manipulation of host's gene expression".

11:25 – 11:45 Coffee break

Session 6: Applications: Bioprocesses and Biotechnology.

Chairman: Antonio Granell

11:45 – 12:05 Eladio Barrio, Universitat de València. "Comparative transcriptomics highlight differences in glycerol metabolism among *Sacchacromyces cerevisiae* species and their hybrids"

12:05 – 12:25 Manuel Cánovas, Universidad de Murcia. "The carbón/energy flow in the *E. coli* central metabolism: the TCA/glyoxylate shunt/overflow metabolism".

12:25 – 12:45 Néstor V. Torres. Universidad de La Laguna. "A Systems Biology approach to analyze the signaling pathway involved in the induction of the L-carnitine biosynthesis in *E. coli* cultures".

12:45 – 13:05 José M. Franco-Zorrilla, CNB-CSIC. "Development of novel experimental strategies for the analysis of plant transcriptomes".

13:05 – 14:00 Wrapping up and conclusions.

14:00 – 15:30 Lunch

15:45 – 17:00 Reunión con Julio Barbas, representante del MICINN.

Posters

1. P. Agudelo-Romero, P. Carbonell, F. de la Iglesia, J. Carrera, G. Rodrigo, A. Jaramillo, M.A. Pérez-Amador & S.F. Elena. IBMCP-CSIC and École Polytechnique. "Changes in the gene expression profile of *Arabidopsis thaliana* after infection with *Tobacco etch virus*".
2. P. Arense, M.R. Foulquie, V. Bernal, C. Bernal, A. Sevilla, M. Cánovas & J.L. Iborra. Universidad de Murcia, Universiteit Brussel and Inbionova Biotech SL. "Genetic engineering of the L-carnitine and central metabolisms of *Escherichia coli*".
3. C. Bernal, A. Sevilla, P. Arense, M. Cánovas & J.L. Iborra. Inbionova Biotech SL and Universidad de Murica. "*In silico* model of the mitocondrial metabolism in cardia cell undergoing alterations in the ATP synthesis".

4. S.C. Cerezo, J.M. Pastor, S. Renilla, T. Fuhrer, V. Bernal, U. Sauer, J.L. Iborra & M. Cánovas. Universidad de Murcia and ETH Zürich. "Acetate overflow role in central metabolism in *Escherichia coli*".
5. M. Dies, À. Robert-Moreno, B. Alsina & J. Villà-Freixa. Universitat Pompeu Fabra. "Modelling early inner ear development in vertebrates".
6. A. Fernández, A. Granell & the ESPSOL partners, IBMCP-CSIC. "An outsider looking deep inside: is it posible to do Systems Biology in the tomato?".
7. Gómez-Garrido, A. L. García de Lomana, M. Hernández-Sánchez, P. Rué-Queralt & J. Villà-Freixa. Universitat Pompeu Fabra. "ByoDyn: unifying computational methods for biochemical models".
8. D. V. Guebel, M. Cánovas & N. V. Torres. Universidades de La Laguna y de Murcia. "Analysis of the *Escherichia coli* response to glycerol pulse in continuous, high-cell density cultura using a multivariate approach".
9. J. A. Hormiga, J. Vera, I. Frías & N. V. Torres. Universidad de La Laguna. "Growth and ligninolytic system production dynamics of the *Phanerochaete chrysosporium* fungus. A modeling and optimization approach".
10. M.A. Molina, A.J. Molina, E. Duque, A. García, J. de la Torre, A. Segura & J.L. Ramos. EEZ-CSIC. "Biological and *in silico* tools to analyze genomes".
11. J. M. Pastor, S. C. Cerezo, S. Renilla, V. Bernal, M. Cánovas & J. L. Iborra, Universidad de Murcia. "Effect of glyoxylate shunt associated genes deletion on the central metabolism energy balance of *Escherichia coli*".
12. J.E. Pérez-Ortín, Universitat de València. "Genomics of mRNA turnover".
13. S. Renilla, T. Fuhrer, J.M. Pastor, S.C. Cerezo, V. Bernal, J.L. Iborra, U. Sauer & M. Cánovas. Universidad de Murcia and ETH Zürich. "Transcriptional regulation of the glyoxylate shunt in *Escherichia coli*".
14. A. Reyes-Palomares, R. Montañez, A. del Real-Chicharro, O. Chniber, A. Kerzazi, I. Navas-Delgado, M.A. Medina, J. Adeana-Montes, & F. Sánchez-Jiménez. Universidad de Málaga. "Systems Biology metabolic modeling assistant (SBMM): An ontology-based tool to integrate metabolic data for kinetic modeling".
15. D. Roche, J. Serra, X. Rovira & J. Giraldó. Universitat Autònoma de Barcelonoa. "Evolutioanry computing appliucations: a genetic algorithm for curve fitting".
16. G. Rodrigo, J. Carrera, A. Jaramillo & S.F. Elena. IBMCP-CSIC and École Polytechnique. "Modeling and optimization of the interaction between RNA silencing pathway and viral suppressors of silencing".
17. X. Rovira, D. Roche, J. Serra, J. Kniazeff, J.P. Pin & J. Giraldó. Universitat Autònoma de Barcelona and CNRS. "Mathematical modelling of metabotropic glutamate receptors function".
18. P. Ruè, J. Villà-Feixa & K. Burrage. Universitat Pompeu Fabra. "Extended stability domain leaping methods for fast approximate stochastic simulation of chemically reacting systems".
19. A. Sevilla, C. Bernal, P. Arense, J.L. Iborra & M. Cánovas. Universidad de Murcia and Inbionova Biotech SL. "Signalling model of *E. coli* L-carnitine metabolism for impairing the glucose catabolite repression".

20. M. Tortajada, F. Llaneras & J. Picó, Universidad Politécnica de Valencia. "Constraint-based modelling applied to heterologous protein production with *P. pastoris*".
21. Vargas, M. Argandoña, M. Reina-Bueno, J. Rodríguez-Moya & J.J. Nieto. Universidad de Sevilla. "Development of Systems Biology of *Chromohalobacter salexigens* to overproduce biostabilizers with versatile applications".
22. M. Weber, Universitat de Barcelona. "Stochastic description of the quorum sensing regulatory network in *Vibrio fisheri*".

Session 1: Modeling and optimization

(Re)constructing realities in Systems Biology: the Jacobian way

Wolkenhauer, O.
Systems Biology and Bioinformatics, University of Rostock, 18051 Rostock, Germany.

I shall introduce systems biology as the art of translating diagrams into mathematical models and then formalize this idea in terms of the reconstruction of rate equations from the Jacobian matrix. It turns out that the relationship between rate equations and the Jacobian requires a subtle mathematical assumption, which has implications on the interpretation of causal entailment in the underlying biological system.

This result raises a question about the role and possibly a principle limitation of ordinary differential equations as representations of biochemical reaction networks.

A global optimization strategy for investigating operating design principles in metabolism: adaptive response of yeast to heat shock

Sorribas, A.[1], Guillén-Gosálbez, G.[2], Jiménez, L.[2], Vilaprinyo, E.[1], Alves, R.[1]
[1]*Departament de Ciències Mèdiques Bàsiques, Universitat de Lleida, 25008 Lleida, Spain.*
[2]*Departament d'Enginyeria Química, Universitat Rovira i Virgili, 43007 Tarragona, Spain.*

Understanding the relationship between gene expression changes, enzyme activity shifts, and the corresponding physiological adaptive response of organisms to environmental changes is crucial in explaining how cells cope with stress. For example, adaptation of yeast to heat shock involves a characteristic profile of changes to the expression levels of genes coding for enzymes of the glycolytic pathway and some of its branches. The experimental determination of changes in gene expression profiles provides a descriptive picture of the adaptive response to stress. However, it does not explain why a particular profile is selected for any given response. In a previous work [1, 2], the adaptive response of yeast to heat shock was investigated and different physiological constraints were identified to shape the observed response [2]. In this work, an intensive computational approach was used to test a large number of hypothetical scenarios. Each of these scenarios was evaluated by computing different performance criteria. Finally, a reduced set of admissible cases was selected. This set, that includes the actual experimental results, is defined as the one compatible with all the considered performance criteria.

Extension of this approach to more complex cases is not straightforward, as the intensive computation involved is prohibitive. In this work, we propose a novel optimization strategy that solves this limitation. We cast the problem as a mixed-integer nonlinear programming (MINLP) problem, in which the pathways involved in heat shock response of yeast are modeled using the Generalized Mass Action (GMA) strategy within the power-law formalism [1]. Such a mathematical representation includes a set of terms that add non convexities into the model. Thus, to guarantee the global optimality of the solutions found, we introduce a global optimization technique

that is based on the outer approximation for global optimization algorithm developed by Bergamini *et al*. [3].

The proposed approach allowed us to identify the small subset of gene expression profiles that lead to effective physiological responses after heat shock. The experimentally observed transcriptional changes in response to heat shock belong to this set and can be explained by quantitative design principles at the physiological level that ultimately constrain changes in gene expression.

Our theoretical approach suggests a method for understanding the combined effect of changes in the expression of multiple genes on the activity of metabolic pathways, and consequently on the adaptation of cellular metabolism to heat shock. This method can be a valuable tool for investigating the evolution of adaptive responses in cell metabolism.

1. Voit, E.O., Radivoyevitch, T. (2000) *Bioinformatics* **16**: 1023–1037.
2. Vilaprinyo, E., Alves, R., Sorribas, A. (2006) *BMC Bioinformatics*. **7**: 184.
3 Bergamini, M.L., Grossmann, I.E., Scenna, N., Aguirre, P. (2008) *Comp. Chem. Engineer*. **32**: 477–493.

Elementary flux modes and optimization

Podhorski, A.[1], Planes, F.J.[1], de Figuereido, L.F.[2], Rubio, A.[1], Beasley, J.E.[3], Schuster, S.[2]
[1]*CEIT and TECNUN, University of Navarra, Spain*
[2]*Bioinformatics Department, Friedrich-Schiller-University, Jena, Germany*
[3]*Mathematical Sciences, Brunel University, UK*

In the post-genomic era elementary flux modes represent a key concept to analyze metabolic networks from a pathway-oriented perspective [1]. Despite their early formulation [2], the computation of the full set of elementary flux modes in large-sized metabolic networks still constitutes a challenging issue to meet. A summary of the different algorithms proposed to carry out this task can be found in [3]. Based on the work of Beasley and Planes [4] we here illustrate that the full set of elementary flux modes can be enumerated via mixed-integer linear programming. Technically, our approach produces elementary flux modes in increasing number of reactions by sequentially solving an optimization problem. Though our procedure is not particularly efficient for large-sized metabolic networks, it is much more flexible. It can be applied to calculate the elementary flux modes satisfying a given criteria without having to calculate all the solutions first, as typically done by current methods. This greatly speeds up the computations by allowing to focus only on that part of the solution space that is of interest. To illustrate the scope of our approach, we here consider two different cases, namely modes in a given length range and ATP producers modes. Our analysis shows that our mathematical approach can be an effective tool to explore the capabilities of metabolic networks, including those at the genome-scale.

1. Schuster, S., Fell, D.A., Dandekar, D. (2000) *Nat. Biotechnol*. **18**: 326-332.
2. Schuster, S., Hilgetag, C. (1994) *J. Biol. Syst*. **2**: 165-182.

3. Klamt, S., Gagneur, J., Von Kamp, A. (2005) *IEE Proc. Syst. Biol.* **152:** 249-55.
4. Beasley, J.E., Planes, F.J. (2007) *Bioinformatics,* **23**: 92-98.

Modeling protein dilution experiments: stochastic effects

Buceta, J.
The SIMBIOSYS Group, Barcelona Science Park, 08208 Barcelona, Spain.

Recent experiments have studied protein expression/regulation at the single cell level. Simply stated, cells are "filled" with a protein's repressor molecule that dilutes out due to cell growth and division. As a consequence, protein expression can be tracked as a function of time (i.e. as a function of the repressor concentration). Protein and repressor levels are characterized by fluorescence microscopy (CFP and YFP resp.) thus allowing the quantification of the relation between repressor levels and the protein production rate: the gene regulatory function. The latter is crucial for understanding and model gene regulatory networks. Herein we present a modeling approach towards this system. Thus, we propose a system of equations that describes, at the single cell level, the production rate of the protein as a function of the concentration of its repressor and the interplay of the protein operator sites. By means of a Krames Moyal expansion we are able to formally derive an effective Langevin description that account for the protein dynamics inside a given cell. In addition, cell cycle effects are taken into account. Our results show protein regulation and production as experimentally measured. We also provide evidence that cooperativity between protein operator sites (repressor binding sites) is required for obtaining the observed gene regulatory function. Importantly, we show that the autocorrelation of the protein production rate presents a resonant effect as a function of the "amount of stochasticity" in the cell cycle: there is an optimal value of the stochastic component of the cell life time that synchronizes (coordinates) protein production within the cell population.

Optimization and optimality in Systems Biology

Banga, J.R.
Instituto de Investigaciones Marinas, CSIC, 36208 Vigo, Spain.

Optimization aims to make a system or design as effective or functional as possible. Mathematical optimization methods are widely used in engineering, economics and science.

This talk is focused on applications of mathematical optimization in computational systems biology. Examples are given illustrating the use of optimization methods in topics including optimal model building, optimality in biochemical metabolic networks, optimization of metabolic engineering and synthetic biology. Finally, several perspectives for future research will be outlined.

Suboptimality in Systems Biology

Pérez–Escudero, A., de Polavieja, G.G.
Instituto Cajal, CSIC, 28002 Madrid, Spain.

Optimality theory has been one of the major predictive frameworks in biology, from the genetic code to behavior. However, biological systems are suboptimal and optimality theory can thus be a very poor predictor for many of the components in a system. Here we show that stochastic optimization, a generalization of optimization theory, predicts the actual suboptimal structure of biological systems. Using stochastic optimization, we predict the structure of diverse systems, including neuroanatomy in *C. elegans* and chemical fluxes in the metabolic networks of *E. coli*. We thus propose stochastic optimization as a predictive framework for Systems Biology.

Session 2: New analytical tools

Computational metabolic engineering

Hatzimanikatis, V.
Laboratory of Computational Systems Biotechnology. EPF Lausanne, 1015 Lausanne, Switzerland.

A possibilistic framework for metabolic flux analysis

Llaneras, F., Sala, A., Picó, J.
Instituto de Automática e Informática Industrial, Universidad Politécnica de Valencia, 46022, Valencia, Spain.

A metabolic network can be seen as a set of constraints that encloses the whole range of pseudo-state behaviours —or flux distributions— that can be achieved by a cell. Traditional and 13C metabolic flux analysis incorporate measurements as additional constraints in order to determine the complete flux distribution. A difficulty that arises when applying these techniques is that the intrinsic uncertainty of the measurements is translated into the determined fluxes in a non-trivial way. Several methods have been proposed to deal with this phenomenon, such as the use of an interval representation of fluxes or the determination of probabilistic confidence intervals.

We introduce a variant of metabolic flux analysis under a possibilistic framework. The approach is based on a reinterpretation of the *consistent causal reasoning paradigm* as an equivalent problem of optimization subject to equality and inequality constraints, which may be interpreted in possibilistic terms.

The methodology incorporates several interesting features: (a) It considers the uncertainty of the measurements to compute a range of possible values —with different degrees of possibility— for each estimated flux. This is more reliable than a unique value and more informative than an interval. (b) The uncertainty of the measurements —and even the constraints— can be characterized in a very flexible way. For instance, it is not necessary to assume that the measurement errors are normally distributed. (c) The methodology is of use even if there is a scarcity of measured fluxes. (d) The flux estimation problem is solved by efficient linear and quadratic programming tools, which are able to cope with large-scale metabolic networks. The procedure is illustrated by the estimation of the metabolic fluxes during a batch fermentation of *C. glutamicum* and a cultivation of CHO cells.

aGEM: an integrative system for the analysis of spatial-temporal gene expression information

Jiménez-Lozano, N., Segura, J., Macías, J.R., Carazo, J.M.
Centro Nacional de Biotecnología, CSIC, 28049 Madrid, Spain.

The work presented here describes the "anatomic Gene Expresssion Mapping (aGEM)" Platform, a development conceived to integrate phenotypic information from spatial and temporal distribution of expressed genes in whole organisms. The aGEM Platform has been built extending the Distributed Annotation System (DAS) protocol that was originally designed to share genome annotations over the WWW. DAS is a client-server system in which a single client integrates information from multiple distributed servers.

The aGEM Platform provides information to answer three main questions: (1) Which are the genes that are expressed in a given anatomical component? (2) In which anatomical structures a given gene (or set of genes) is expressed? And (3) is there any correlation among these findings? Currently this Platform includes several well-known mouse resources (EMAGE, GXD and GENSAT), hosting gene expression data obtained from *in situ* techniques together with a broad set of image derived annotations. The Platform access is through a friendly and intuitive display: http://bioweb.cnb.uam.es/VisualGenomics/aGEM.html.

Casting genomic data into systems biology concepts

Dopazo, J.
Department of Bioinformatics and Genomics, CIPF, 46012 Valencia, Spain.

The ultimate goal of any genome-scale experiment is to provide a functional interpretation of the results, relating the available genomic information to the hypotheses that originated the experiment. Initially, this interpretation has been made on a pre-selection of relevant genes, based on the experimental values, followed by the study of the enrichment in some functional properties. Nevertheless, functional enrichment methods demonstrated to have a flaw: the first step of gene selection results too stringent given that the cooperation among genes (within the modules aimed to find) was implicitly (and paradoxically) ignored. The assumption that modules of genes related by relevant biological properties (functionality, co-regulation, chromosomal location, physical interaction between proteins, etc.), and not the genes alone, are the real actors of the cell biology dynamics, lead to the development of new procedures implicitly closer to systems biology concepts. Such procedures, generically known as gene set methods, have successfully been used to analyze transcriptomic and large-scale genotyping experiments as well as to test other different genome-scale hypothesis in other fields such as phylogenomics. The use of functional or regulatory modules has, however, some limitations that deserve to be commented.

Symmetric monoidal (bi)categories with feedback and biological networks

Pareja-Tobes, E., Manrique, M., Tobes, R. Pareja, E.
Era7 Bioinformatics, BIC Granada, 18100 Granada, Spain.

Most biological systems are concurrent and distributed, and it could be argued that their complexity overly exceeds that of non-living systems. It is clear that modelling frameworks capable of managing concurrency, distribution, and specific characteristics of biological systems such as the presence of relationships not only between their

components but also between the processes, would lead to both novel predictions and deeper conceptual understanding. As an answer for these needs, we propose the framework of symmetric monoidal (bi)categories with feedback, introduced by Walters [1, 2] for the modelling of concurrent distributed systems. The main features of our setting are:

* The possibility of modelling relations between processes, "processes between processes": nodes, arrows between nodes, and 2-arrows between these arrows.
* Graphical calculus along the lines of string diagrams in traced symmetric monoidal categories [3].
* Observing different aspects of the system becomes applying forgetful functors from the same model to adequate categories.
* Features and relations from metabolic, genetic and signaling networks can be integrated within the same system, with no need of complete information about all the components of the biological system at hand.

We will introduce this framework by means of an example, then go through a comparison between our approach and current (qualitative) modelling frameworks such as Petri nets [4], or the general framework proposed by Wolkenhauer in [5].

1. Katis, P., Sabadini, N., Walters, R. (1997) *J. Pure Appl. Algebra* **115**: 141–178.
2. Katis, P., Sabadini, N., Walters, R. (2000) *Rendiconti del Circolo Matematico di Palermo Serie II, Suppl* **63**: 123–156.
3. Joyal, A., Street, R., Verity, D. (1996) *Math. Proc. Camb. Phil. Soc.* **119**: 447–468.
4. Breitling, R., Gilbert, D., Heiner, M., Orton, R. (2008) *Briefings in Bioinformatics*
5. Wolkenhauer, O. Hofmeyr, J. (2007) *J. Theor. Biol.* **246**: 461–476.

Session 3: Systems Biology of microorganisms

Systems Biology: a physiological journey from El Greco to Matisse - the art of science

Booth, I.
School of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, Scotland.

Systems Biology can trace its origins to the work of Darwin and *The Origin of Species*. My own preoccupation with this old (new) science originates in my work as a bioenergeticist studying the quantitative aspects of lactose transport in *E. coli*. In this presentation, I will try to explain our approach to systems analysis of methylglyoxal detoxification and KefB activation. This linked system of enzymes and transporters protect *E. coli* cells from the toxicity of this electrophile. In this lecture I will explain how this system works and what new insights have been provided by the systems approaches that we have developed.

The regulatory duties of the phosphotransferase system (PTS^{Ntr}): A metabolic flux analysis of *Pseudomonas putida*.

Chavarría, M.[1], Kleijn, R.J.[2], Sauer, U.[2], Pflüger, K.[1] de Lorenzo, V.[1]
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The environmental bacterium *Pseudomonas putida* is known for its metabolic versatility and endurance to various types of stresses. This requires various layers of control to coordinate the expression of specific genes to the overall physiology of the cell. One prevalent physiological sensor to this end is the phosphoenolpyruvate-carbohydrate phosphotransferase transport system (PTS). Apart from the classical phosphoenolpyruvate: carbohydrate phosphotransferase system (PTS), many prokaryotes harbor also a PTS branch that is not involved in carbohydrate traffic, but participates in regulation of some metabolic processes in a fashion dependent on their phosphorylation state [1]. The genome of *Pseudomonas putida* KT2440 encodes to two distinct classes of PTS systems: (i) PTS^{Fru}, a classical PTS system responsible for fructose uptake and (ii) PTS^{Ntr}, the so-called nitrogen-metabolic PTS, which lacks any connection to sugar traffic. However, under certain metabolic conditions an *in vivo* cross talk between the two systems can be observed [2-4]. Furthermore, the PTS^{Ntr} is involved in the regulation of polyhydroxyalkanoate (PHA) accumulation and C-source repression of *m*-xylene catabolism [2, 5]. In order to explore the gross functional depth of PTS^{Ntr} in the metabolism of this bacterium, we analyzed the metabolic fluxes of isogenic strains bearing non-polar directed mutations in each of the corresponding PTS genes. This fluxomic analysis revealed that the PTS^{Ntr} controls the connection of pyruvate to the TCA cycle by downregulating the pyruvate shunt, which bypasses malate dehydrogenase in the TCA cycle. It is believed that excess C is removed *via* this shunt in *P. putida*. On the contrary, the distinct breakdown of the carbon flow between

the competing Entner-Doudoroff route, the pentose phosphate pathway, and the ordinary Embden-Meyerhof-Parnas glycolysis was not affected in any mutant. All examined metabolic effects of the PTS^{Ntr} could be traced to the sole presence/absence of PtsN ($EIIA^{Ntr}$), regardless of its phosphorylation state. In a next step, we verified the regulatory influence of PtsN on the pyruvate shunt by measuring the activity of the malic enzyme and the pyruvate carboxylase. The differences in the net fluxes and enzymatic activities, together with the observation that the PTS^{Ntr} influences expression of the TOL biodegradation pathway, biofilm formation, and the intracellular accumulation of polyhydroxyalkanoates, provide evidence for the multiple regulatory functions for the PtsN ($EIIA^{Ntr}$) protein of *P. putida*.

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Systems Biology of *Mycoplasma*, a minimal organism

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In our group we aim at reaching a quantitative understanding of a living organism. Our target is *Mycoplasma pneumoniae*, a bacteria from the Mollicutes class bearing only 686 genes. The project encompasses several strategies: a) metabolism is reconstructed and metabolic fluxes are obtained by carbon-13 tracing; b) transcriptional network is revealed by high-throughput DNA microarray and tiling array analysis; c) regulatory components are identified by gain of function experiments. Moreover the relationship between the environment and genetic control of metabolism is determined by the combination of these approaches.

The project will benefit from a structural genomics project aimed at determining the structures of all *M. pneumoniae* proteins ("Structural proteome of Mycoplasma", Berkeley), from existing extensive previous work (Professor Dr. Herrmann, from ZMBH, is our advisor) and from the participation in the context of the CRG/EMBL partnership of a consortium involving the EMBL structural and computational biology programme. Currently all proteins of the organism are been tagged for pull down to identify all the protein complexes, electron microscopy is done on these complexes to obtain 3D shapes which will be fitted inside EM 3D tomograms of the bacteria at 40-50 A resolution.

We expect that this level of knowledge will set up the basis to perform synthetic biology of *M. pneumoniae*, a much simpler organism than other bacterial models, such as *E. coli*. Ultimately a computer model will be generated integrating all this information, which should be accurate enough to be predictive.

Biochemical and proteomic approaches to the study of potassium homeostasis in *Saccharomyces cerevisiae*

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Ion homeostasis is a basic process in living cells and potassium is the most abundant intracellular cation. In the model yeast *Saccharomyces cerevisiae*, K^+ homeostasis is the final result of the functioning of potassium influx (Trk1 and Trk2) and potassium efflux systems that work tightly regulated.

In the context of the SysMo program and the Translucent project, we follow biochemical and proteomic approaches in the study of potassium homeostasis in *S. cerevisiae.*

From a biochemical point of view, we are studying the cation content, fluxes and regulatory pathways in *Saccharomyces*. By using a standard medium (YNB potassium free), we have characterised a potassium transport mutant (*trk1,2*) and the corresponding wild type. Potassium requirements and the kinetic characteristics of rubidium (potassium) uptake in cells grown in the newly defined medium have been determined. Moreover adaptation to the high affinity potassium transport mode during starvation and changes in cell volume have also been studied. The final objective is to elaborate simple mathematical models that may allow predicting the behaviour of the cell under different situations of potassium stress.

By using a differential expression proteomics approach we are analyzing changes in the protein profile between wild and double mutant (*trk1,2*) strains, under the following conditions: i) optimal growth potassium concentration (20 mM), exponential and stationary phase; ii) absence or limiting (5 mM) potassium concentration, 30 min, 1, 3, and 5h. The following workflow has been used: i) protein extraction (buffer homogenization of the cells and TCA-acetone precipitation of the proteins); ii) protein separation by two-dimensional (IEF, SDS-PAGE) electrophoresis; iii) Coomassie gel staining, densitometer image capture, and spot quantisation; iv) statistical analysis of the data, and identification of qualitative or quantitative differential spots; v) trypsin digestion of the protein spots; vi) mass spectrometry analysis of the tryptic peptides (MALDI-TOF-TOF); vii) protein identification from MS and MS^2 spectra.

Modeling cation homeostasis in the yeast *Saccharomyces cerevisiae*: a gene discovering and transcriptomic profiling approach

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Maintenance of cation homeostasis is necessary for cell survival. For instance, in the yeast *S. cerevisiae* preservation of appropriate electrochemical gradient across the membrane (involving mainly H^+, K^+ and Na^+ transport) is crucial for nutrient uptake.

This model organism is very well suited for a Systems Biology approach, since the major cation influx and efflux machineries and their regulation in response to cation stress are well characterized, and a variety of genome-wide experimental approaches are available. Work at UAB is being done in the context of a SysMo international project (TRANSLUCENT). Two major medium-term goals have been defined: 1) the identification at genome-wide scale of genes necessary for maintenance of the electrochemical gradient and 2) the generation of transcriptomic profiles in response to altered potassium homeostasis.

A collection of around 4800 haploids mutants was scored for tolerance to 3 different toxic compounds (spermine, hygromycin B and TMA), all three entering the cell driven by the electrochemical gradient. Almost 200 genes have been identified as required for normal tolerance to all three drugs. In addition to known genes (i.e. *TRK1* high-affinity K^+ transporter) we have uncovered many components of the DNA remodeling machinery (ADA and SAGA complex), vacuolar biogenesis and intracellular trafficking (SNARE and vesicle tethering complexes). Among them, so far four mutants (v*am3, vam7, lst7 and yol087c*) have been found to show inhibited growth at limiting potassium (1 mM). Experiments to evaluate membrane potential, K^+ uptake and Trk1 presence at the plasma membrane are underway.

The second approach is based on the generation of transcriptomic profiles in response to altered potassium homeostasis. Preliminary analysis of *trk1,2* mutant shows alteration of specific amino acid biosynthetic pathways, even in the presence of large amounts of K^+. Shifting cells to K^+-free medium triggers a substantial alteration of the expression pattern: genes encoding sulphur- and phosphorus-related metabolism are induced in a time-dependent manner, whereas ribosomal protein-encoding genes are repressed. Microarray data and phenotypic analysis indicates that K+-starved cells suffer a significant degree of oxidative stress.

Work in our laboratory was supported by grant GEN2006-27748-C2-1-E/SYS to JA.

A multi-level approach to the study of heterologous protein production in *Pichia pastoris* under different oxygen conditions

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In a study for functional, structural and regulatory processes involved in the expression of a complex protein under critical environmental conditions, we applied different oxygenation rates for the constitutive expression of an antibody Fab fragment in *Pichia pastoris* cultivations. The recombinant *Pichia pastoris* strain and its control strain were grown in a chemically defined medium in a glucose-limited chemostat. The oxygen set points were changed from high to low air concentrations, resulting in a stepwise reduction of the oxygen concentration in the inlet air from 20.97% to 10.91% and 8,39% (vv^{-1}). For cultures that received less than 20.97% oxygen in the gas stream, air was partially replaced with nitrogen. Interestingly, a 2.5 fold increase of the specific productivity q_P was observed when shifting from respirative to hypoxic growth conditions, which were accompanied by sub product formation (mainly ethanol and a C5 sugar alcohol) and a decrease in biomass yield. These observations gave rise to the development of an optimised fed batch process ensuring a permanent low level of fermentative metabolism, which was tested successfully with three different protein producing strains.

In order to reveal the physiology behind this phenomenon, we have investigated the transcriptional response of *Pichia pastoris* at the different levels of oxygen concentrations using *Pichia pastoris* specific whole genome DNA micro arrays.

This transcriptomic approach, which will provide a more detailed overview of transcriptional regulation patterns under the given experimental conditions, was supplemented by proteomics to check the direct involvement of the gene products at the protein level. We have detected a relative increase in the abundance of proteins involved in general fungal stress response and in glycolysis at low oxygen concentrations, while proteins linked to TCA cycle and glycerolipid metabolism show decreased levels. Furthermore, such impact on the central carbon metabolism has been corroborated at the level of metabolic fluxes by means of ^{13}C-based metabolic flux analyses. In general, our analyses indicate a strong oxygen-dependent expression pattern rather than a protein-expression related pattern for all the genes studied.

Summarized, the omics approaches should help to understand the cellular mechanism that leads to increased product formation at low oxygen and might reveal hitherto unidentified key factors or major pacemakers of efficient protein production.

Session 4: Metabolic engineering and synthetic biology

Computational design of synthetic circuits

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Ultimately, synthetic biology aims at establishing novel, useful biological functions by suitably combining well-characterized parts. Major open issues are: (i) principles of circuit design with standardized parts, and (ii) functional design of robust performance. In the first area, we developed a method for the design of genetic circuits with composable parts. Through integration into ProMoT (Process Modeling Tool), we realized a "drag and drop" tool to build genetic circuits as in engineering design. In the second area, we focus on minimal biochemical oscillators as an example. In particular, we are interested in the robustness - the insensitivity to perturbations – of design alternatives to understand design principles of complex synthetic networks.

Neutrality and robustness in evo-devo

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Embryonic development is defined by the hierarchical dynamical process that translates genetic information (genotype) into a spatial gene expression pattern (phenotype) providing the positional information for the correct unfolding of the organism. The nature and evolutionary implications of genotype-phenotype mapping still remain key topics in evolutionary developmental biology (evo-devo). We have explored here issues of neutrality, robustness and diversity in evo-devo by means of a simple model of gene regulatory networks. The small size of the system allowed an exhaustive analysis of the entire fitness landscape and the extent of its neutrality. This analysis shows that evolution leads to a class of robust genetic networks with an expression pattern characteristic of lateral inhibition. This class is a repertoire of distinct implementations of this key developmental process, the diversity of whom provides valuable clues about its underlying causal principles.

Engineering a minimum photoautotrophic cell: the case of *Synechococcus elongatus* PCC7492

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Members of the so called picocyanobacteria from the genus *Prochlorococcus* and *Synechococcus* are among the most abundant photosynthetic bacteria in the oceans. These marine photosynthetic bacteria have being studied extensively at the genomic level. Comparative analyses from eleven strains of *Synechococcus sp.* have revealed genomic islands of hipervariability related to niche adaptation, whereas twelve genomes from *Prochlorococcus sp.* have being used to predict a core genome coding for all functions for a viable cell. The phylogenetically related *Synechococcus elongatus*, is a freshwater unicellular rod-shaped obligate photoautotrophic cyanobacterium. With a genome of approximately 2.7 Mb and two plasmids (8 and 46 kb) *S. elongatus* was the first cyanobacterium demonstrated to be reliably transformable by exogenously added DNA. Two strains from *S. elongatus* (PCC7492 and PCC6301) have being sequenced to date. With the aim to engineer a reduced genome we have made a comparative analysis of *S. elongatus* genome with all other sequenced cyanobacteria. The results are discussed in terms of essential versus dispensable genes and the core genome for this species. Here we present the first steps towards the engineering of a reduced genome for a photosynthetic autotrophic genetically malleable prokaryote.

Bacterial two component systems

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Bacterial two-component systems (TCS) are key signal transduction networks regulating global responses to environmental change. Environmental signals may modulate the phosphorylation state of sensor kinases (SK). The phosphorylated SK transfers the phosphate to its cognate response regulator (RR), which causes physiological response to the signal. Frequently, the SK is bifunctional and, when unphosphorylated, it is also capable of dephosphorylating the RR. The phosphatase activity may also be modulated by environmental signals. We constructed mathematical models to examine the steady-state and kinetic properties of the network.

Mathematical modeling reveals that a) bifunctionality has physiological implications that can justify its selection, and b) the TCS can show bistable behavior for a given range of parameter values if unphosphorylated SK and RR form a dead-end complex that prevents SK autophosphorylation.

Additionally, for bistability to exist the major dephosphorylation flux of the RR must not depend on the unphosphorylated SK. Structural modeling and published affinity studies suggest that the unphosphorylated SK EnvZ and the RR OmpR form a dead-end complex. However, bistability is not possible because the dephosphorylation of OmpR approximately P is mainly done by unphosphorylated EnvZ. Some implications of this potential bistability in the design of a TCS network will be discussed.

Session 5: Applications: Biomedicine

Methods in quantitative microbial metabolomics: how to get the right numbers?

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Obtaining reliable and accurate experimental data is a crucial in Systems Biology. If detailed and extensive mathematical models of biological systems are based on erroneous data, the insights and predictions obtained from those models are of little use. It is therefore of prime importance that the experimental procedures to generate these data are thoroughly checked for their reliability and reproducibility and that they are improved if they do not meet the required criteria. This presentation focuses on the development and application of an experimental platform for quantitative microbial metabolomics, whereby it is shown how the applied experimental and analytical procedures, e.g. the cultivation of the micro-organisms, rapid sampling, fast quenching, metabolite extraction and analytical procedures can be evaluated and optimized. Subsequently, the optimized procedures were applied in research aimed at unraveling the metabolic regulation of central metabolism in different micro organisms, i.e. *Saccharomyces cerevisiae*, *Penicilium chrysogenum* and *Escherichia coli*, thereby showing that often the experimental procedures have to be adapted for a particular micro organism.

A Systems Biology approach to multifactorial diseases

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Several techniques as DNA sequencing, expression arrays, and proteomic and metabolomic experiments have provided us a large amount of new information that cannot be easily interpreted. The integration of all these *in vivo* information in models is likely to be the most interesting tool to understand and to complete an overview picture of the cellular processes. Metabolic profile is the end point of the signaling events, where changes caused by diseases may be reflected. Using data from the different –omics, incubation with ^{13}C labeled substrates and isotopomer analysis in selected metabolite pools, and appropriate software developed in our laboratory to estimate dynamic flux distribution among the metabolic network we are able to identify the main steps that control a metabolic pathway, which may be used as new therapeutical targets. We are applying this approach to understand metabolic adaptations accompanying different multifactorial diseases as cancer, diabetes and chronic obstructive pulmonary disease (COPD). Applying these strategies we identify the maintenance of pentose phosphate cycle oxidative and nonoxidative unbalance to be critical for cancer cell survival and vulnerable to chemotherapeutic intervention. Additionally, we used Metabolic Control Analysis (MCA) to identify the main enzymes controlling ribose-5-P synthesis and to plan combined target strategies.

Finally, we validated the obtained strategies using specific inhibitors. This strategy results of great interest in imminent applications for the study of other multifactorial diseases. In particular, we are applying this strategy to achieve a better understanding of glucose metabolic network to design interventions at a metabolic level in diabetes and COPD. This new principle for rational drug design originates from the integrative, systems biology approach of understanding cell function and opens new ways to develop novel treatments for diseases as diabetes or COPD.

Global connectivity and activity distributions in cellular networks

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Various molecular interaction networks have been claimed to follow power-law decay for the global connectivity distribution. It has been proposed that there may be underlying generative models that explain the heavy-tailed behavior by self-reinforcement processes such classical or hierarchical scale-free network models. We have analyzed a comprehensive data set of protein-protein and transcriptional regulatory interaction networks in yeast, an *E. coli* metabolic network, and gene activity profiles for different metabolic states in *E. coli* and yeast. We show that in all cases the networks have a heavy-tailed distribution, but most of them present significant differences from a power-law model according to a stringent statistical test. Those few data sets that have a statistically significant fit with a power-law model follow equally well other distributions. Thus, while our analysis supports that both global connectivity interaction networks and activity distributions are heavy-tailed, they are not generally described by a specific distribution model leaving space for further inferences on generative models.

Research of trehalose in pathogenic yeasts as a useful model of infectivity for plants and mammals

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The non-reducing disaccharide trehalose is widely conserved in nature from archeons to bacteria and eukaryotic organisms (fungi, invertebrates or plants), but excluding mammals. In fungi, trehalose plays a dual role as reserve carbohydrate and as protectant of cell integrity against a set of nutritional and environmental perturbations (stress). Furthermore, trehalose has been considered a virulence factor in pathogenic fungi. Thus, the genes involved in the biosynthetic pathway, namely *TPS1* (trehalose-6P synthase) and *TPS2* (trehalose-6P phosphatase) increase the resistance of *Candida albicans* (the main pathogenic yeast in humans) against the niche-specific reactive oxygen species produced by phagocytic cells. In fact, the homozygous mutants disrupted in those genes undergo a great loss of infectivity. Likewise, in some phytopathogenic fungi (*Rhizoctonia solani* and *Magnaporthe grisea*), trehalose synthesis is required for primary infection of plants.

Here, we have studied the interaction of both *tps1Δ*/*tps1Δ* and *tps2Δ*/*tps2Δ* mutants, which are deficient in trehalose synthesis with human macrophages (coming from peripheral blood of healthy donors and the cell line THP-1) and murine macrophages (coming from the intraperitoneal cavity of Swiss mice and the cell line J774). *tps2Δ* but not *tps1Δ* cells exhibited a phenotype of thermosensitivity. Through the use of fluorescence microscopy after staining with phaloidin and DAPI, it was observed that both *tps1Δ* and *tps2Δ* strains were completely engulfed after 2 hours of co-culture (10:1 yeast/macrophage ratio). The presence of *C. albicans* cells growing actively triggered a clear pro-inflammatory response, quantified by means of ELISA and identified by an increase of TNF-α in the medium. This response was stimulated as long as yeast cells were phagocytosed by the macrophages, although its intensity was lower in *tps1Δ* cells respect to the parental CAI.4 strain. These data confirm the existence of structural defects in the cell wall, provoked by the pleiotropic effect caused by the double disruption of *TPS1* gene.

Under all the yeast:macrophage ratios examined (1:1, 5:1 and 10:1), the mutants were always more susceptible to phagocytic lysis than the wild type strains. Previous stimulation of macrophages with LPS and IFN-γ increased the difference in sensitivity to lysis in the *tps2Δ* mutant, this pre-treatment being indispensable to record it in the *tps1Δ* mutant. Collectively, our results support a direct role of trehalose biosynthesis in the mechanism of tissue infection and colonization evolved by *C. albicans*. Thus, the compounds able to antagonize synthesis and/or hydrolysis of trehalose must have a potential interest in antifungal chemotherapy. We propose to utilize this system as a model for general studies on infectivity in humans.

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Comparative transcriptomic analysis between virulent and non virulent strains of *S. cerevisiae* in fresh blood.

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S. cerevisiae is widely distributed in nature and is responsible for the alcoholic fermentation during wine, beer, cider, sake and bread making. In addition to the importance of *S. cerevisiae* in fermentative processes, the beneficial role in health of this yeast is well-known because of its nutritional value and a series of biofunctional properties, a reason why it is also used as a dietary supplement. On the other hand, *S. cerevisiae var. boulardii* (a therapeutic *S. cerevisiae* strain marketed as Ultralevura®) is at the moment used as biotherapeutic agent, but has recently been described in literature its use as a probiotic in foods or drinks in a similar way to that of bacterial probiotics. The revision of medical literature indicates there has been an increasing incidence of fungemia by *S. cerevisiae* in the last years, many of them produced by *S. cerevisiae var. boulardii*. Consequently, at the moment *S. cerevisiae* is considered, along with other yeasts, to fall within the group of emergent opportunistic pathogens. Previous study based on phenotypic characteristics associated with virulence and using murine models of systemic infection we observed the pathogenic potential of some food isolates and also a high number of clinical isolate. In the present work we have study virulent and avirulent strains of *S. cerevisiae* by global expression in fresh blood to detected special pathway related with the virulent traits of *S. cerevisiae*. The strains were incubated in fresh blood and samples were taken at 0', 15', 30', 60' and 90'. The transcriptomic analysis shows several differences:

- Five thioredoxin peroxidases are induced in virulent strains but not in the control. Also one glutathione peroxidase is induced and is not in the control.
- Many genes related to sulphur-methionine metabolism and transports are induced in virulent strains and only one in the control.
- Glyoxylate pathway genes are induced in lab strain but is not induced in one virulent and repressed in the other.

These preliminary data can indicate an increased ability of the virulent strains to counteract the oxidative stress caused by macrophage oxidative burst attack. Also these results can indicate an enhanced adaptation of virulent strains to growth in human extracellular media.

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Virus adaptation by manipulation of host's gene expression

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Viruses adapt to their hosts by evading defense mechanisms and taking over cellular metabolism for their own benefit. Alterations in cell metabolism as well as side-effects of antiviral responses contribute to symptoms development and virulence. Sometimes, a virus may spill over from its usual host species into a novel one, where usually will fail to successfully infect and further transmit to new host. However, in some cases, the virus transmits and persists after fixing beneficial mutations that allow for a better

exploitation of the new host. This situation would represent a case for a new emerging virus. Here we report results from an evolution experiment in which a plant virus was allowed to infect and evolve on a naïve host. After 17 serial passages, the viral genome has accumulated only five changes, three of which were non-synonymous. An amino acid substitution in the viral VPg protein was responsible for the appearance of symptoms, whereas one substitution in the viral P3 protein the epistatically contributed to exacerbate severity. DNA microarray analyses show that the evolved and ancestral viruses affect the global patterns of host gene expression in radically different ways. A major difference is that genes involved in stress and pathogen response are not activated upon infection with the evolved virus, suggesting that selection has favored viral strategies to escape from host defenses.

Session 6: Applications: Bioprocesses and Biotechnology

A new strategy for assessing global sensitivity in biochemical networks

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Models of biochemical networks are one of the main research tools in systems biology. Modelling and simulation are useful as a means of integrating experimental data from diverse sources and check their consistency, and as a means to understand and gain insight about biochemical networks and physiological phenomena. This understanding passes through linking parameters to their effect on the biochemical variables. The knowledge that can be derived from this has impact on experimental design, drug target selection, and the study of disease states in general. However, the values of many parameters of a network model have often not been determined or are not identifiable due to technical experimental difficulties or other constraints.

I will first describe existing approaches to assess the importance of model parameters on biochemical variables of interest. One of these is based on sensitivity analysis but the classical ways in which it has been applied in biochemistry (metabolic control analysis and biochemical systems theory) constitute local methods, meaning that they are only valid the around the nominal value of the parameters. This means that parameters need to be known with considerable accuracy. In modern systems biology it has become obvious that one does not have the luxury of knowing all such values. Therefore a different approach is needed and one way is to carry out sensitivity analysis over a wide range of values for all parameters, but this analysis is computationally intractable for most realistic models. Another approach is to employ global sensitivity analysis (GSA) which is normally based on random sampling methods, and while tractable is computationally very expensive. Here I present an efficient alternative approach that involves using numerical optimizing methods to search a wide region of parameter space of a given model to determine the maximum and minimum values of its sensitivity coefficients. An example relevant for drug development is presented to demonstrate the strategy using the software COPASI.

Trancriptomic analysis revealed that the species of the genus *Saccharomyces* and the interspecific hybrids present differences in the glycerol metabolism.

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S. *cerevisiae* is the predominant species responsible of the alcohol fermentation; however other closely related species, belonging to the so-called *Saccharomyces sensu stricto* complex (new *Saccharomyces* genus) may have an important role during

fermentation processes. Alcohol fermentations are not the best suited medium for the development of *Saccharomyces* yeasts. Since wine fermentations were developed by the first agricultural societies, *Saccharomyces* wine strains were unconsciously selected due to their ability to ferment substrates with high sugar contents to become adapted to the fermentation conditions predominant in each region. This way, yeast strains from the Mediterranean region tolerate high sugar and alcohol contents and high fermentation temperatures, whereas, Central European strains are adapted to lower sugar and alcohol contents, and lower fermentation temperatures. Along their evolution, yeasts have suffered diverse selective processes to become adapted to the fermentation conditions. These events led to the unconscious 'domestication' of the 'industrial' yeasts possessing physiological and genetic properties very different from that exhibited by wild yeasts [1, 2]. Different molecular mechanisms, such as gene duplication, aneuploidy, poliploidy, chromosomal rearrangements, interspecific hybridization, etc., were involved in the generation of the evolutionary novelties selected during domestication that allowed the adaptation of yeasts to the fermentation processes [2].

In the case of the *Saccharomyces sensu stricto* yeasts, one of the most interesting mechanisms involved in their adaptation to industrial processes is the generation of interspecific hybrids. Interspecific hybridization generates new gene combinations of potential adaptive value conferring, under fluctuating or intermediate environmental conditions, selective advantages to the hybrids with respect to their parental species. In recent studies, new hybrids resulting from the hybridization between *S. cerevisiae* and *S. kudriavzevii* have been described among wine [3, 4] and brewing [5] strains. The oenological characterization of these wine hybrids showed that they exhibit several better winemaking properties than the parent species; they acquired the ethanol tolerance and the ability to grow in media containing high levels of sugar from the *S. cerevisiae* parent and improved growth at lower temperatures from the *S. kudriavzevii* parent [6, 7]. This characteristic is of great value due to the current tendency in the wineries to perform wine fermentation at low temperatures to obtain fruity and aromatic wines. However, many selected *S. cerevisiae* strains are not well adapted to such low temperature conditions that may lead to inadequate yeast growth and slow or stuck fermentations. The DNA chip analyses of yeast gene expression during microvinification, indicate that hybrids exhibit a significant higher expression of the genes involved in the glycerol synthesis (*GPP1* y *2);* in the adaptation (stress response) to low temperatures due to an over-expression of the genes related with the synthesis of ergosterol (*ERG1* and *ERG3, ERG11, ERG26*) and of cold-shock genes (*TIR1*, *TIR2, PAU3, PDR5, YHB1*and *TIP1*). In addition, we also detected an over-expression of some genes encoding amino acid transporters (*AGP1*, *FUR4*, *RHB1*, *BAP3).*

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The carbon-energy flow in the *E. coli* central metabolism: The TCA/glyoxylate shunt/overflow metabolism

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The use of microorganisms as cell-factories requires unraveling the mechanisms which regulate central metabolism as a result of metabolic evolution. The major drawbacks for the use of *Escherichia coli* for the biosynthesis of primary and secondary metabolites and heterologous proteins are the excretion of carbon as acetate, the unbalance of redox state and the regeneration of the pool of HS-CoA. The glyoxylate shunt (GS) allows the assimilation of acetate and its use for biosynthetic pathways while the acetate metabolism acts as an overflow metabolism as well as part of the anaerobic metabolism producing ATP. In this work the transcriptional regulation of the *aceBAK* operon by deleting the *aceA, aceK* and *iclR* genes and the removal of the acetate metabolism by deleting the *acs* and *pta* genes were analyzed. The enzymes aceB (malate synthase) and aceA (isocitrate lyase) of the GS and aceK (isocitrate dehydrogenase phosphatase kinase) modulates the activity of the Tricarboxylic Acids Cycle (TCA), while acs (acetyl-CoA synthase) and pta (phosphotransacetylase) are responsible of the *Pta-ack* and *acs* operons expression. This part of the central metabolism depends on transcriptional regulator factors such as FruR, IHF, IclR, CRP, ArcA and Fnr.

In order to analyze the regulatory mechanisms that control carbon flux towards GS and the acetate overflow under glucose and acetate as the carbon sources, absolute internal fluxes, relative expression levels of 25 genes, internal and external metabolites and key enzymatic activities were determined in *E. coli* BW25113 and the mutants, *ΔaceA ΔaceK, ΔiclR, Δacs* and *Δpta*. Results obtained show that an altered regulation of GS and TCA node at isocitrate level and the acetate metabolism removal can improve the assimilation of carbon source. Furthermore, this resulted in the establishment of improved new connections between the transcriptome, enzymome, metabolome and fluxome of the cell for the use in biotechnological processes.

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A Systems Biology approach to analyze the signaling pathway involved in the induction of the L-carnitine biosynthesis in *E. coli* cultures

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L-(-)-carnitine can be synthesized from some cheap, raw waste bioprecursors such as crotonobetaine and D-(+)-carnitine. This biotransformation is carried out by the enzymes encoded by the caiTABCDE and fixABCEX operons, both regulated by the cAMP receptor proteins. Therefore, the biotransformation must be performed on non phosphorylated sugars such as glycerol, since glucose inhibits the cAMP synthesis and the carnitine metabolism at the expression level. However, the use of glucose as the carbon source might be more efficient than glycerol since the L-(-)-carnitine biosynthesis is ATP dependent.

In this work we have made use of the available information on the signaling structure involved in the biotransformation process to build up a mathematical model aiming to design biotechnological strategies to abolish the glucose inhibiting effect and thus to improve the biotransformation yield.

Based on experimental data we have built up a model using a GMA power-law representation, which was used as platform to make predictive simulations. Thereby, we could assess the consistency of the regulatory structure of the overall process. The model parameters were estimated from a time series experimental measurements by means of an algorithm previously adapted and optimized for power-law models. The model was subsequently checked for quality by comparing its predictions with the experimental behavior observed in new, different experimental settings and through perturbation analysis aimed to test the robustness of the model.

The model description its helping in advance our understanding of the system's kinetic and regulatory signal features and to the design of an optimized biotechnological setup for the continuous production of L-(-)-carnitine.

Development of novel experimental strategies for the analysis of plant transcriptomes

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Systems Biology strategies on the analysis of transcriptomes allows integration of experimental data from multiple sources, helping modelling of biological events. The development of new tools for the analysis of whole genomes is essential to enhance our understanding on transcriptomic dynamics. In this communication we will focus on two different genomic strategies intended to improve our knowledge of the regulation of plant transcriptomes.

First of these strategies comes from our interest to identify specific DNA-protein interactions in order to accurately predict the DNA-targets of transcription factors

(TFs). With this aim, we have developed an universal double stranded DNA microarray containing all possible nucleotide combinations for 11 bp motifs, allowing identification of specific DNA motifs for virtually any TF. This methodology has been successfully applied for the identification of the DNA-binding sequence of 6 different TFs from plants and humans, and is currently being applied to the identification of the DNA-binding site of 50 families of plant TFs. In addition to TFs, it can be potentially applied to any sequence-specific DNA-binding protein.

The second strategy is aimed to investigate the impact of miRNA (and other small RNAs) in post-transcriptional regulation of target mRNAs. In plants, miRNAs guide cleavage of their corresponding targets by means of (nearly) perfect complementarity. To date, approximately 200 miRNAs have been identified in Arabidopsis, but deep sequencing of small RNAs suggests that the actual number of miRNAs may be higher. We have developed a methodology for discovery of miRNA-targets at the genome level based on molecular enrichment of targets and microarray hybridisations, providing a genomic landscape of miRNA-guided degradome in plants.

Changes in the gene expression profile of *Arabidopsis thaliana* after infection with *Tobacco etch virus*.

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Tobacco etch potyvirus (TEV) has been extensively used as model system for the study of positive-sense RNA virus infecting plants. TEV ability to infect *Arabidopsis thaliana* varies among ecotypes. In this study, changes in gene expression of *A. thaliana* ecotype L*er* infected with TEV have been explored using long-oligonucleotide arrays. *A. thaliana* L*er* is a susceptible host that allows systemic movement, although the viral load is low and syndrome induced ranges from asymptomatic to mild. Gene expression profiles were monitored in whole plants 21 days post-inoculation (dpi). Microarrays contained 26,173 protein-coding genes and 87 miRNAs.

Expression analysis identified 1727 genes that displayed significant and consistent changes in expression levels either up or down, in infected plants. Identified TEV-responsive genes encode a diverse array of functional categories that include responses to biotic (such as the systemic acquired resistance pathway and hypersensitive responses) and abiotic stresses (droughtness, salinity, temperature, and wounding). The expression of many different transcription factors was also significantly affected, including members of the R2R3-MYB family and ABA-inducible TFs. In concordance with several other plant and animal viruses, the expression of heat-shock proteins (HSP) was also increased. Finally, we have associated functional GO categories with KEGG biochemical pathways, and found that many of the altered biological functions are controlled by changes in basal metabolism.

TEV infection significantly impacts a wide array of cellular processes, in particular, stress-response pathways, including the systemic acquired resistance and hypersensitive responses. However, many of the observed alterations may represent a global response to viral infection rather than being specific of TEV.

Genetic engineering of the L-carnitine and central metabolisms of *Escherichia coli*

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The production of L-carnitine takes place under anaerobic conditions and depends on diverse environmental stimuli, such as the absence of oxygen and PTS sugars. Therefore, the aim of this work was to investigate the effect of two single-gene knocking mutations on the carnitine metabolism in *Escherichia coli* under aerobic conditions and grown in a medium rich in glucose. The targets were the promoters of *caiF* and the operon *cai*, both of which were removed and replaced by others constitutive promoters (p8 and p37), which lacked the binding centers for proteins of global regulation (FNR and CRP), avoiding their repressive activity on the levels of enzymes of the metabolism of L-carnitine. The carnitine production was measured in batch reactor to study the expression of the genes belonging to the carnitine metabolism and the metabolites of the central metabolism were analyze by HPLC to follow the changes produced in the cellular physiology. These engineered strains allowed the production of L-carnitine in aerobiosis conditions and to determine the changes produced in the central metabolism, with which it was possible to establish that the effect of the CRP transcription factor is not totally at genetic level, since differences in the central metabolism of the new strains with respect to the wild type were observed. Therefore, one fact that still remains to be solved is the level of action of PTS sugars in *E. coli*. This work is being undertaken by our group.

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In silico model of the mitochondrial metabolism in cardiac cell undergoing alterations in the ATP synthesis

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The ability to predict clinical efficacy *in silico* could save diagnostic and pharmaceutical prescription time and resources, and ultimately lead to more targeted and personalized therapies. In this work we have studied physiological and pathological conditions in cardiac cell mitochondria by means of the use of *in silico* simulations. To carry out this study, a model of the mitochondrial metabolism has been developed. This model contains glycolysis/pyruvate metabolism, carnitine system, Krebs cycle, fatty acids β-oxidation, cardiolipin synthesis, electron transport chain coupled to ATP production/H^+ translocation systems, ROS detoxification and ATP/ADP, oxygen, CO_2, Pi/ H^+ transporters. The major function of mitochondria is to produce ATP by a process called oxidative phosphorylation. This process involves an electron transport chain (*ETC*) that generates an electrochemical gradient of protons that drives the synthesis of ATP from ADP and P_i via the ATP synthase (F_0F_1). There are several mitochondrial metabolic alterations where the synthesis of ATP could be decreased, like the Barth Syndrome, the sudden death, the hypoglycemia and other ETC alterations. This study concerns the behaviour of cardiac cell mitochondria metabolism undergoing different alterations in the ATP synthesis using *in silico* simulations and the

results obtained showed that the model predicted adequately the experimental data. We can conclude that a System Biology approach can be used in the clinical diagnosis and treatment of cardiac progressive disorders.

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Acetate overflow role in central metabolism in *Escherichia coli*

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Since the central metabolism in *Escherichia coli* controls both the production of metabolites associated with cell growth and the heterologous proteins production machinery, both of biotechnological interest, the aim of this work was the characterization of *Escherichia coli* BW25113 *knockout* strains, in acetate assimilation and excretion pathways, in order to deeper our knowledge of the involvement of these enzymes in the metabolic flux distribution.

E. coli BW25113 (control) and its phosphotransacetylase (*pta*) *knock-out* mutant, were cultured in batch reactors using minimal medium with glucose as carbon sources. Biomass and metabolite production, the variation of extracellular pH redox ($NADH/NAD^+$) and energetic (ATP) states and key enzymatic activities were followed during cultivation. Grown on glucose as the carbon source, Δ*pta* produced mainly lactate. Moreover, Δ*pta* in aerobiosis and with glucose as carbon source reached a higher biomass yield than the control strain, due to the assimilation of lactate showing a diauxic effect. This mutant showed, unexpectedly, higher ATP levels.

From a biotechnological point of view, a 75% lower acetate production and better assimilation of carbon sources, makes *Δpta* interesting for its use as a protein factory. However, at an adaptive level, the deficiency in this pathway makes the strain more sensitive to environmental changes. Moreover, the function of *pta* is likely to be more important since its deletion deregulates central metabolism.

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Modeling early inner ear development in vertebrates

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The inner ear is a sensory organ responsible for the perception of sound and balance in vertebrates. It derives from the otic placode: a thickening of the ectoderm adjacent to the hindbrain formed early in development that is composed of few hundred cells.

Experimental observations show that neural fate occurs at the otic placode stage, when two well differentiated domains - proneural and non-neural- arise [1, 2]. In this work we try to unravel the origin of the neurogenic specification by modelling the effect of the presence/absence of Fgf8 on the expression of Lmx1b, a key determinant of the regionalization process.

We propose a model considering six species (Fgf8, Fgf10, Sox3, Ngn1, Tbx1, Lmx1b) for which qRT-PCR data is obtained. The model parameters are estimated from the experimental data by automatically exploring a reasonable biological rank of values around a literature curated set of starting parameters. Parameter estimation and numerical integration of the model are carried out with Byodyn (http://cbbl.imim.es/ByoDyn). Knock down *in silico* experiments for the decrease of Fgf8 activity are performed based on the best results from the optimization process in order to contrast our model with in situ hybridization data of the effect of such mutation. A discussion about the qualitative trends the model provides is performed, as well as a critical assessment of literature data for the kinetics underlying the gene regulation model.

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An outsider looking deep inside: Is it possible to do SB in the tomato?

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The generation of large datasets in functional genomics projects offers an opportunity to explore what a SB look at those datasets may reveal about the underlying biological structure. In the case of tomato, a model system for fleshy fruit development, the most recent emphasis has been on the identification of genes and gene regions associated to fruit quality traits. In those instances quality has been inferred from the levels of certain metabolites such as sugars, organic acids, healthy compounds such as carotenoids (b- carotene and lycopene), and aroma volatiles. The availability of microarray platforms that allow the expression profiling of good part of the tomato genome and its use in well defined and phenotyped populations (such as large collections of recombinant inbred lines or collections of introgression lines that basically introgress one genome into the other in defined fragments) enables the identification not only of metabolite QTLs but also expression QTLs. These large datasets (comprising phenotypic and genotypic information) can be interrogated using different tools to identify correlations, associations between the different data type. We will present what we would like to do and hopefully find someone to help us do it.

ByoDyn: Unifying computational methods for biochemical models

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It is becoming a routine task to build models of increasing complexity on a given biochemical network or pathway of interest. One of the main problems in building such models is the determination of the parameters underlying each modelled process. ByoDyn has been designed to provide an easily extendable computational framework to estimate and analyze parameters in highly uncharacterized models.

ByoDyn includes a set of tools to 1) integrate ordinary differential equations (ODEs), including systems with events, rules (differential algebraic equations, DAE) and delays (DDE) and simulate stochastic processes using Gillespie's SSA and _ leap methods, built from a given biological model; 2) globally optimize the parameters that fit the provided experimental information and evaluate the sensitivity of the model with respect to the different parameters; and 3) include the sensitivity of the parameters in an optimal experimental design pipeline. The program makes use of external software to solve some problems, providing a Python binding schema that allows the user to easily implement new software in the desired calculation protocol. The program benefits from its interface with the SBML library, which ensures communication with other existing tools in the field.

ByoDyn (GPL) is a Python based program built on top of several open source libraries, PORT FORTRAN, SciPy and libSBML, and it provides easy binders to OpenModelica and Octave. The program has been tested in Linux Fedora and in Mac OS X platforms, having been parallelized in a Platform LSF grid environment and on a Slurm+MOAB scheduling system. ByoDyn code and complete documentation is available at http://cbbl.imim.es/ByoDyn. Finally, a fully functional version can be accessed on-line in the same web site, with different solutions for highly demanding simulations.

Analysis of the *Escherichia coli* response to glycerol pulse in continuous, high-cell density culture using a multivariate approach

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Pulse experiments in continuous-culture are a valuable tool in microbial physiology research. However, inferences become difficult when the cell response is followed by monitoring many biochemical variables or when several types of perturbations are compared. Moreover, there is no objective criterion to delimit the time-window, so that the recorded responses will render valid inferences. Hence, we have investigated the capability of a multivariate approach to deal with complex data from a previously

described series of pulse experiments. Data are concerned with twelve biochemical variables that were monitored when an anaerobic, steady-continuous culture of *E. coli* O74K74 was disturbed by six types of pulses (glycerol, fumarate, acetate, crotonobetaine, hypersaline plus high-glycerol basal medium and crotonobetaine plus hypersaline basal medium). Our analysis determined the instantaneous uptake rate for the pulsed metabolite (Dynamical Chemical-Balances), reduced the multivariate observations to one response curve (Principal Component Analysis) and determined the optimal time-window (Cluster Analysis). Finally, input-output data were filtered (Orthogonal Signal Correction) while both blocks were mathematically connected (Partial Least-squares Regression). This systematic approach allowed us to detect several relevant patterns not previously revealed: i) Glycerol uptake rate did not follow a Michaelian kinetics but showed a biphasic dependence on glycerol concentration; noticeably, net uptake decreased 136-fold despite the high availability of glycerol in the milieu. ii) The structure of the bacterial response changed during time the glycerol-disturbance lasted (2 hours), hence analyses had to be limited to the early response (time from 0 to 5 min). iii) By mathematically relating the input (glycerol uptake rate) with the output (twelve biochemical responses) it was possible to identify which of the monitored variables were primary targets of the glycerol disturbance (namely: ATP, formate, acetyl-CoA synthase, isocitrate dehydrogenase and isocitrate lyase), which were secondarily responsive (ethanol) and those that were independent (acetate, carnitine, lactate and NADH/NAD ratio). Identification was achieved even though all the analyzed variables were affected by the pulse. iv) Some variables exhibited uncorrelated dynamics despite their close functional relationship (ATP and NADH/NAD ratio, ethanol and lactate; carnitine and the crotonobetaine hydratase complex; acetate and the enzymes phosphotransacetylase, acetyl-CoA synthase and isocitrate lyase). The results are discussed in terms of *E. coli* transcriptional control, and it is concluded that glycerol pulse produces a stressing effect. The consequent activation of the polyamine-dependent mechanisms involved in such stressing effect provides a unified explanation for how glycerol uptake is down-regulated in the presence of high glycerol availability and how acetate can be produced without de novo biosynthesis.

Growth and ligninolytic system production dynamics of the *Phanerochaete chrysosporium* fungus. A modelling and optimization approach

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The well-documented ability to degrade lignin and a variety of complex chemicals showed by the white-rot fungus *Phanerochaete chrysosporium* as made it the subject of many studies in areas of environmental concern, including pulp bioleaching and

bioremediation technologies. However, until now, most of the work in this field has been focused on the ligninolytic sub-system but, due to the great complexity of the involved processes; less progress has been made in understanding the biochemical regulatory structure that could explain growth dynamics, the substrate utilization and the ligninolytic system production itself. In this work we want to tackle this problem from the perspectives and approaches of Systems Biology, which have been shown to be effective in the case of complex systems. We will use a top-down approach to the construction of this model aiming to identify the cellular subsystems that play a major role in the whole process.

We have investigated growth dynamics, substrate consumption and lignin peroxidase production of the *P. Chrysosporium* wild type under a set of definite culture conditions. Based on data gathered from different authors and in our own experimental determinations, we built a model using a GMA power-law representation, which was used as platform to make predictive simulations. Thereby, we could assess the consistency of some current assumptions about the regulatory structure of the overall process. The model parameters were estimated from a time series experimental measurements by means of an algorithm previously adapted and optimized for power-law models. The model was subsequently checked for quality by comparing its predictions with the experimental behavior observed in new, different experimental settings and through perturbation analysis aimed to test the robustness of the model. Hence, the model showed to be able to predict the dynamics of two critical variables such as biomass and lignin peroxidase activity when in conditions of nutrient deprivation and after pulses of veratryl alcohol. Moreover, it successfully predicts the evolution of the variables during both, the active growth phase and after the deprivation shock. The close agreement between the predicted and observed behavior and the advanced understanding of its kinetic structure and regulatory features provides the necessary background for the design of a biotechnological setup designed for the continuous production of the ligninolityc system and its optimization.

Biological and *in silico* tools to analyze genomes

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Pseudomonas putida KT2440 is a saprophytic bacterium used as a model system to study biodegradation and interactions of a nonsymbiotic microorganism with plants. This strain, whose genome was sequenced in 2002 [1], is a nutritional opportunist and a paradigm of metabolically versatile microorganisms [2]. The initial genome annotation suggested 5,420 open reading frames (ORFs) with a set of almost 2,100 genes identified as the core genome of *Pseudomonas* [3]. This indicates that the number of noncore genes of KT2440 (considered to contribute to the fitness and versatility in its natural habitat) is larger than the number of core genes. The construction of mutant libraries of this strain can provide clues of its lifestyle in certain ecological niches and reveal its specific properties. So far, the library under construction in our lab contains more than 2,000

different mutants. The combination of bioinformatics and experimental data, allows researchers to reconstruct metabolic pathways; analysis of mutants in each one of the proposed steps could reveal important information about the pathway. This approach has allowed us to characterize in detail the glucose degradation pathway [4, 5] and to study the biosynthetic pathways of aromatic aminoacids (this poster).

As part of our bioinformatic activities, we have constructed a web-based tool (Provalidator) that helps to design and validate generalized profiles to define protein families in prokaryotes. Provalidator combines nearly-full automation of the profile-building construction step and the search for family members in all available databases. The tool is freely available at www.bacTregulators.org. As proof of concept we constructed a profile that best defines the MerR family of transcriptional regulators and another profile that defines and classifies root-nodulation-cell-division (RND) multidrug efflux pumps.

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Effect of glyoxylate shunt associated genes deletion on the central metabolism energy balance of *Escherichia coli*

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Since the central metabolism in *Escherichia coli* controls not only the production of metabolites associated with growth but the production of heterologous proteins as well, both of biotechnological interest, the aim of this work is the characterization of *Escherichia coli* BW25113 strains knockout in the glyoxylate shunt, in order to increase the knowledge of the involvement of these enzymes in the metabolic fluxes.

A knockout lacking the first enzyme of the pathway (Δ*aceA*) was employed as negative control, while the other two mutants Δ*iclR* y Δ*aceK*, lacked regulator genes. IclR acts as a repressor of the pathway, whereas the *aceK* product is a dual modulator. Biomass and metabolite production of the mutants and the wild type, were measured in several batch reactors. Extracellular pH variation and energetic and redox states were assessed too, along with nine key enzymatic activities involved in several pathways of central metabolism (glyoxylate shunt, Krebs cycle, glycolysis, fermentation, pentose-phosphate pathway and acetate uptake), at different growth stages. When growing on glucose as sole carbon source, Δ*aceA* mutant showed an increased $NADH/NAD^+$ ratio through all growth phases. This fact, along with diminished ATP levels in all mutants compared to wild type strain, could point to a role of glyoxylate pathway in energy and redox balance, even on this carbon source.

On acetate, *aceA* mutant was unable to grow, whereas Δ*aceK* exhibited a slower growth rate and higher ATP level than the control strain.

Results showed a new putative role of the glyoxylate bypass, which seems to rise when growing aerobically on glucose, despite its low activity under these conditions. Further studies focusing on the involvement of this fact in central metabolism flux distribution will help optimizing biotechnological processes using *E. coli* as a biofactory.

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Genomics of mRNA turnover

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Most studies on eukaryotic gene regulation have focused on mature mRNA levels. Nevertheless, the steady-state mRNA level is the result of two opposing biological processes: transcription and degradation, both of which can be important points to regulate gene expression. It is now possible to determine the transcription and degradation rates (TR and DR), as well as the mRNA amount, for each gene using DNA chip technologies. In this way, each individual contribution to gene expression can be analyzed. We have developed several new techniques to evaluate both TR and DR in the yeast *Saccharomyces cerevisiae*. They will be described in detail and their potential drawbacks discussed. The comparison of data obtained by different techniques is giving some clues on the mechanisms of transcription and post-transcriptional mechanisms in eukaryotes.

Transcriptional regulation of the glyoxylate shunt in *Escherichia coli*

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The complex regulation of catabolic and anabolic processes makes possible the adaptation of microorganisms to different environments. The use of microorganisms as biofactories requires unraveling the mechanisms which regulate central metabolism.

The major drawback for the use of *Escherichia coli* for the biosynthesis of primary and secondary metabolites and heterologous proteins is the excretion of carbon as acetate. The glyoxylate shunt (GS) allows the assimilation of acetate and its use for biosynthetic pathways. In this work the transcriptional regulation of the *aceBAK* operon was analyzed. AceB (malate synthase) and AceA (isocitrate lyase) are enzymes of GS while AceK (isocitrate dehydrogenase phosphatase kinase) modulates the

activity of the Tricarboxylic Acids Cycle (TCA). The control of the operon expression depends on transcriptional regulator factors such as FruR, IHF, IclR, CRP, ArcA y Fur.

In order to analyze the regulatory mechanisms that control carbon flux towards GS under glucose excess and limitation, absolute internal fluxes and relative expression levels of 25 genes were determined in *E. coli* BW25113 and the mutant *ΔiclR*. Results obtained show that an altered regulation of GS and TCA node at isocitrate level can improve the assimilation of carbon source. Furthermore, this resulted in the establishment of improved new connections between the transcriptome and the fluxome of the cell for the use in biotechnological processes.

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Systems Biology Metabolic Modeling Assistant (SBMM): An ontology-based tool to integrate metabolic data for kinetic modeling

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The dynamic analysis of biochemical reactions networks is an essential approach to understand the intrinsic complexity of biological systems. In fact, development of biocomputational methods for metabolic modeling is a very active field at present. The systems biology platform has progressed successfully to offer a structured standard of biological information (SBML.org). The result has been reflected in the performance of software for analysis of biological networks (i.e.: Cytoscape) and dynamical behavior of biochemical reactions (i.e.: Copasi, Celldesigner). This advance is correlated to the growth rate of biological information and the reorganization of the data service architectures. For metabolic modeling, diverse information is required, from characteristics of the reaction participants, up to information on the global network structure and dynamics. One of the more recognized problems for efficient kinetic modeling is the lack knowledge of kinetic parameters. To alleviate this problem, very valuable kinetic data repositories were built and are being improving (i.e., Brenda and Sabiork). In the present communication, we describe the Systems Biology Metabolic Modeling assistant (SBMM assistant), an application designed to facilitate metabolic modeling process. SBMM assistant, works on the bases of an ontology-based mediator developed to integrate data from KEGG, CHEBI, BRENDA and SABIORK. SBMM assistant is characterized by the following features: It is an SBML-compatible and friendly tool able to guide to the novel or experienced user to capture, enrich, generate and visualize biological networks, to make basic queries on enzymic kinetics and regulation, and to annotate this information following the MIRIAM specifications. Semantic-web technologies have been claimed to be applied specifically to solve the present shortcomings in the workflow of metabolic modeling (Lee *et at.*, 2008). In this

sense SBMM assistant is an example of such a specific application, having the aim to act as a complementary tool (assistant) for metabolic modeling analysis programs. Thus, it is not only able to capture, enrich and store the information in models, but also helps the cross-talk among the different resources and tools in a friendly way. The facts provide novel capabilities to SBMM with respect to other previously reported applications.

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Evolutionary computing applications: a genetic algorithm for curve fitting

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The Hill equation is likely the most used model for curve-fitting in pharmacological research [1]. However, it presents a possible drawback as it cannot account for asymmetric concentration-effect curves [2]. The Richards function solves this problem by including an additional parameter (*s*). Hill and Richards models are nested, being the Hill function a particular case of Richards equation if the s parameter is equal to one. A value of *s* different from one allows a theoretical curve to display an asymmetric shape. Regretfully, it has been found that, in a number of cases, the Richards function performs deficiently in curve fitting if gradient nonlinear regression is used [3]. The reason for that may lay in the strong correlation found between some of the parameters of the Richards model. This correlation can affect the reliability of the location, slope, and symmetry parameters yielding nonsensical values, very large errors or even failing to converge [2].

A genetic algorithm (GA) can be a useful methodology for the determination of the parameters of difficult fitting problems [4]. In particular, this technique avoids the sensitivity of local optima to the initial estimates supplied to the nonlinear regression procedure.

This study presents a GA approach for the estimation of Richards function parameters. I n the cases tested, our GA provided similar or better estimates than the nonlinear regression method when the latter performed reasonably well or badly, respectively. Because the assessment of asymmetry may be important both for accurate estimation of empirical pharmacological parameters and for the mechanistic analysis of those biological systems where asymmetry is an intrinsic and relevant feature[2], our approach could be a possible choice in those situations in which gradient nonlinear regression (in particular of Richards function) is unsatisfactory.

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Modeling and optimization of the interaction between RNA silencing pathway and viral suppressors of silencing

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The underlying working principle in RNA silencing relies on the auto-repressive action triggered by the intracellular presence of double-stranded RNA (dsRNA). RNA-dependent RNA polymerases (RdRp) replicate single-stranded RNA (ssRNA). This ssRNA can be aberrant or external (from viruses or viroids). During replication, dsRNA intermediates are formed and a cellular molecule, called DICER, degrades these dsRNA into 21 to 24 units called small interfering RNAs (siRNA). Subsequently, the cellular RNA-induced silencing complex (RISC) loads these small RNAs resulting in the active form RISC*. Then, the sequence complementarity between the loaded siRNA and the viral genomic RNA guides RISC*, resulting in the cleavage of the target RNA. Furthermore, in a secondary cycle of amplification, the siRNAs can be used as primers to generate more siRNAs. siRNAs can be moved from cell-to-cell immunizing new cells against infection. Given the properties of the RNA silencing pathway (specificity and amplification), it represents a sort of immune system in plants.

However, viruses have evolved strategies to escape for silencing surveillance while promoting their own replication. Several viruses encode suppressor proteins which interact with elements of the silencing pathway and block it. Several possible viral strategies exist, depending upon which is the target of the suppressor: DICER, siRNA, RISC, or cell-to-cell diffusion of siRNAs. Also, the viroids also can escape from RNA silencing by means of their highly folded structure.

Here, we present a mathematical study of the silencing mechanism and develop models that incorporate different suppressor activities. These models are very important to unveil defense strategies and design principles of genetic systems. In addition, we have performed a parametrical optimization of this pathway to explore in which regions of parameter space hosts can evolve optimal strategies able of silencing different viruses and also whether viruses can evolve capable of surviving in different hosts.

Mathematical modelling of metabotropic glutamate receptors function

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Metabotropic glutamate receptors (mGluRs) are constitutive dimers. Protomers belonging to these receptors are composed of three structural domains, the extracellular Venus flytrap (VFT) domain where agonists and antagonists bind, the cysteine-rich domain (CRD) that interconnects the VFT to the transmembrane heptahelical domain (HD), and HD. mGluRs constitute a complex system both structurally: a dimer of three-domain subunits, which encompasses a number of states in equilibrium (open and closed for the VFT and inactive and active for the HD) and mechanistically: the propensity of activation of the HD dimer depends on the state (open-open, closed-open and closed-closed) of the VFT [1].

Mathematical models can be of prime importance in cell signalling studies for the analysis of complex mechanisms. In this communication, a mathematical model for the function of mGluRs will be shown [2]. Because of the high number of parameters included in the model, curve fitting parameter optimization was performed by an in-house stochastic evolutionary algorithm rather than by classical gradient nonlinear regression.

Quantitative characterization of the functional response was made, including analytical expressions for agonist efficacy and potency. The model allowed the distinction between binding (to open states) cooperativity and induction (of closed states) cooperativity, being the latter the condition that characterizes agonism. To provide the model with parameters with biophysical meaning, a functional published study [3] involving wild-type and single mutated mGluR was reanalysed [2]. The analysis quantitatively described the main effects of receptor mutation on the mechanistic parameters responsible for the functional response.

This study suggests that our model may be useful not only for mGluRs but for any other system that contains an extracellular orthosteric binding domain and a transmembrane allosteric functional domain.

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Extended stability domain leaping methods for fast approximate stochastic simulation of chemically reacting systems

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(Bio)chemically reacting systems involving low populated species of molecules are not correctly modelled by ordinary differential equations. This is due to uncertainty on what reaction is triggered at each time step and how to evaluate such time step in problems that, in addition, are sometimes stiff. Randomness in chemical events may play a critical role in biological processes. Stochastic simulations methods for these systems have therefore attracted much recent interest. Gillespie's stochastic simulation

algorithm (SSA) is the de facto method used to sample from the grand probability described by the chemical master equation (CME) associated with the reactive process of interest, at the cost of very expensive calculations. Tau-leap methods make use of the Poisson distribution for triggered events to efficiently accelerate the stochastic simulation algorithm. We investigate possible extensions of the standard Tau leap method to bigger step sizes, an essential improvement for simulating big systems.

Signalling model of *E. coli* L-carnitine metabolism for impairing the glucose catabolite repression

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Several studies have shown that the addition of glucose leads to total suppression of the carnitine metabolism as a consequence of the catabolite repression [1-3]. In some circumstances, as occurs in the carnitine metabolism, it is essential to overcome the catabolite repression of glucose since, firstly, ATP has been demonstrated to be essential in this process [4-6] and, secondly, glucose has a higher ATP yield than glycerol in anaerobic conditions since glycerol (per average carbon atom) is more reduced than carbohydrates [7] and consequently produces a higher amount of NADH which should be regenerated by means of reduced fermentation products as acetate or ethanol. As a consequence, less ATP will be synthesized through acetate formation in the fermentation process than with glucose. This fact is the principal cause to decide to use glucose as the carbon source instead of glycerol in order to increase the productivity of the bioprocess.

Herein, we present a signalling approach to improving a secondary metabolism biotransformation process in bacteria. The purpose of this article is to analyze, as an example, the signalling structure of carnitine metabolism, especially the effect of cAMP-CRP complex and to use the resulting knowledge to improve the biotransformation process involved in the L-(-)carnitine biosynthesis. We integrated the cAMP-CRP regulon, glucose and glycerol operons as well as the carnitine metabolism in a model which was validated with experiments using different carbon sources as well as different addition strategies. Furthermore, this model was able to predict the effect of the addition of exogenous cAMP to the reaction medium to check the possibility of impairing the glucose catabolite repression of the carnitine metabolism by its addition. In this model, the cAMP-CRP regulon, glucose and glycerol operons as well as the carnitine metabolism were integrated which was validated with experiments using different carbon sources as well as different addition strategies. Furthermore, this model was able to predict the effect of the addition of exogenous cAMP to the reaction medium to check the possibility of impairing the glucose catabolite repression of the carnitine metabolism by its addition.

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Constraint-based modelling applied to heterologous protein production with *P. pastoris*

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Under the pseudo-state assumption, a metabolic network represents a set of constraints that encloses the whole range of behaviours —flux distributions— that can be achieved by the modelled cell. Within this framework, elementary modes analysis reveals as a powerful tool to assess emergent properties and to direct metabolic engineering. Traditional and 13C metabolic flux analysis incorporate measurements as constraints to determine the actual flux distribution; if these methodologies cannot be applied due to scarcity of measurements, the flux spectrum can be used instead. These techniques have been applied in different canonical biologic models, such as CHO cells and bacteria cultures, but rarely in heterologous protein producing systems. *P. pastoris* is a prominent host for foreign protein production well known for its strong methanol-induced alcohol oxidase promoter.

A simplified metabolic network for *P. pastoris* system has been developed concerned with glycerol and methanol main metabolic pathways, energetic balances, and heterologous protein production. Elementary modes have been used to check that the theoretical capabilities of the network are coherent with *P. pastoris* basic physiology. As a second step, some emergent properties of the network have been analysed. Finally, the flux distributions of *P. pastoris* have been investigated under different experimental conditions. Since, to our best knowledge, complete 13C experiments have not been yet reported and only measurements of extracellular species are available, we extracted several incomplete scenarios from the literature and our own experimental work. Then, the flux spectrum approach was applied to get insight into the flux distribution for each scenario.

Constraint-based modelling has been applied to study a microbial system of industrial interest. The usefulness of the approach has been proved even when intracellular measurements are not available: a combination of extracellular measurements and metabolic network knowledge might provide a valuable insight into the state of the system.

Development of Systems Biology of Chromohalobacter salexigens to overproduce biostabilizers with versatile applications

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The biostabilizers ectoine and hydroxyectoine, synthesized by the halophilic γ-proteobacterium *Chromohalobacter salexigens* in response to osmotic and heat stress [1-4], have biotechnological applications. Ectoine (2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) is used as protecting agent in dermopharmacy formulations and as protein and nucleic acids stabilizer in kits for molecular biology (i.e PCR, immunoassay). Novel areas of application might include, for example, its use to inhibit the amyloid formation associated to neurodegenerative diseases. The 5-hydroxylated derivative of ectoine, hydroxyectoine, has putative applications as stabilizer agent against heat, desiccation, and freezing.

Very recently we have been granted with two Projects (from the Science and Innovation Ministry and Junta de Andalucía) aimed to develop the Systems Biology of *C. salexigens* in order to optimize its metabolic engineering to generate strains that overproduce ectoines. Thus, we will combine experimental techniques with *in silico* analysis to improve ectoine(s) synthesis in general, or hydroxyectoine production in particular, as this is currently a minoritary product. In addition, we will explore the potential of these solutes in Biomedicine as stabilizing agents for cryopreservation of biological material and as neuroprotectants. As a first step, a genome-scale reconstruction of the metabolic network in *C. salexigens* will be generated. Secondly, we will perform a global experimental analysis of the response to hyperosmotic and heat stress in *C. salexigens,* as these are the environmental conditions which trigger ectoine and hydroxyectoine synthesis, respectively. Third, we will build up a dynamic model integrating ectoine metabolism with its genetic regulation structure. This model will be tested to predict adequate strategies in order to improve the mentioned bioprocesses. Finally, we will address the evaluation of the effect of ectoines as (i) antioxidative and neuroprotective agents and (ii) cryoprotectants for biological material (RNA, bacterial cells, hepatocytes and enterocytes).

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Stochastic description of the quorum sensing regulatory network in *Vibrio fisheri*

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Bacteria like the *Vibrio fischeri* are able to sense their own cell density by means of a gene regulatory mechanism combined with chemical communication. The main regulatory pathway includes an autoinducer that diffuses out of the cell and plays the role of the chemical signal. Above a threshold value of concentration for the autoinducer in the medium, the regulatory network activates the production of the luminescent molecule Luciferase. The bacteria can monitor the cell density through the autoinducer concentration and alter their behavior. This process, called quorum sensing (QS), is achieved through an appropriate gene network architecture including two main regulatory proteins, LuxI and LuxR.

We introduce a modeling approach for the QS regulatory network, including a deterministic and a stochastic approach of the N-cells system. The dynamics are modelled by a system of chemical kinetic equations and solutions are simulated with the Gillespie algorithm. The distribution of the steady states is calculated and the influence of parameters in the precision of the QS process is analyzed