1st FEBS Advanced Lecture Course on
Systems Biology:
From Molecules & Modeling to Cells
Gosau, Austria, EU, March 12-18, 2005

(westerhoffgosau3corr998.doc)

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AstraZeneca

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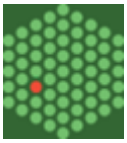

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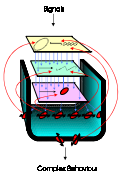
Amsterdam Systems Biology Institute



EML (European Media Laboratory)

GEN-AU GENOMFORSCHUNG IN ÖSTERREICH GENome Research in AUstria

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Willkommen in Gosau, Welkom in Gosau, Welcome to Gosau!

In this snowy environment, we warmly welcome you to the first European Advanced Course on Systems Biology. Around the turn of the previous century, Biology was revolutionized: the sequence of complete genomes became available. Almost immediately many high-throughput, genome-wide analyses sprung up, which will soon enable us also to measure the expression levels of all genes at most levels of the cellular hierarchy. Perhaps never before, there has been such a sense of urgency. Never before did we seem to be so close to knowing how Life functions in terms of the properties of its molecules. Never before could we begin to think of the rational engineering of drugs targeting pathophysiology rather than individual molecules. Yet, when confronted with massive data sets about the molecules of living cells, one tends to get confused rather than illuminated; the function of living cells cannot easily be read from what happens to the molecules. Much of functioning depends on many molecules simultaneously, which engage in complex interactions.

In parallel, biochemistry and biophysics focused more and more on the experimental assessment of molecular interactions. Together with mathematical biochemistry, these disciplines generated new paradigms for understanding how functional properties arise in interactions. These paradigms remained limited however, because not all components of the cellular systems were considered in the analyses, and because most components could not be accessed experimentally.

In 2005 much excitement lies in the synergy of the two above developments: functional genomics gleans from biochemistry, biophysics and mathematical biology how new function arises in nonlinear interactions, whereas the latter three may engage in functional genomics in order to measure all components that are important for the living cell. In fact all these paradigms are now merging into what one might call Integrative Systems Biology. Integrative Systems Biology is here defined as the science investigating how much of the functioning of living organisms comes about in the nonlinear interactions of all their molecules.

From this definition, from the large size of even the smallest genomes, and from the multitude and diversity of nonlinear interactions in Biology, it is readily understood that the challenge that Integrative Systems Biology poses is enormous. This young Science will require so much expertise in both experimental molecular biology and mathematics, in a highly interactive mode, that the way of doing Biology is being revolutionized. As Physics was in the 1950's, Biology is now becoming Big Science, *i.e.* Systems Biology. A new generation of scientists is needed. These scientists should be at ease with both experimental molecular biology and complex mathematics, and with almost anything that is in between. They should also be able to interact strongly and productively with each other, in large teams. This Course is meant to catalyze the formation of this new generation of scientists, from very young Ph D students, but also from established researchers. In this way the course should contribute to the development of science, also for the sake of humanity.

The need for training in Systems Biology was well recognized by our main sponsor, *i.e.* the Federation of European Biochemical Societies (**FEBS**). We appreciate the strong support through the FEBS Advanced Course Committee, in particular its previous chairman **Karel Wirtz**.

The need for training is also recognized by the national European organizations that fund modern biological and medical research. Indeed, the German Ministry for Education and Research (**BMBF**) and the Dutch Organization for Scientific Research, in particular its section on Earth and Life Sciences (**NWO-ALW**), have generously supported the course largely through student registration waivers.

The European Science Foundation (**ESF**), which aims to make the activities of the National European Science Funding Organizations synergize, has likewise given strong support. The ESF engages in a Forward Look study on Systems Biology, which will prepare its final report during the two days following this course, also in Gosau.

The 6th Framework Program of the European Union has funded a similar reconnaissance study on Systems Biology, through a Specific Support Action **EUSYSBIO**. EUSysBio also supports this course, as it will help define the Systems Biology field. A network of Excellence recently funded by the EU and partly directed at Systems Biology, *i.e.* **BioSim** coordinated by **Erik Mosekhilde**, has immediately assimilated this Course into its program of furthering excellent Systems Biology in Europe.

The number of new drugs that reach the market, and the number that thereafter survive, is diminishing. The cost of developing the drugs is becoming astronomical, largely because it is too difficult to choose between the large numbers of promising drug leads at an early stage. The ones that are plagued by 'side effects' and will not interact optimally with their target in the context of the living organism, are identified so late in the process that they absorb most of the budget. The critical issues here are again Systems Biology issues, and modern pharmaceutical companies are engaging strongly in this new field. Two of these, *i.e.*, **AstraZeneca** and **NovoNordisk**, enthusiastically support this meeting, both in terms of its lectures and **AstraZeneca** also in terms of the USB-sticks provided to all participants. Of course, various Software companies engage strongly in Systems Biology, not the least in interaction with these companies and the top Systems Biology Centers (see below). Of these, **Teranode** co-supports the USB sticks and their formatting.

It is unbelievable how ill-defined some of the food is that we enjoy on a daily basis. Both in terms of food safety, and in terms of improvements in their contribution to health, food production methodology is a field that may also be revolved by Systems Biology developments. After all, the production of food by living organisms and its use by human beings, abounds of interacting molecules in the context of living cells. **DSM** and **Purac** are supporting this Course.

The European Journal of Biochemistry (EJB) has been a pillar under Biochemistry in more than one way. First, it has always published scientific articles of high quality and significance. Second, it has always earned much of the money that is used to subsidize FEBS courses. At present, the journal is even more relevant to the emerging field of Systems Biology: (i) it is one of the earliest journals that highlighted the topic, such as through the direct link to the siliconcell model-base (www.siliconcell.net), and (ii) it has just undergone a facelift, becoming the **FEBS Journal** and orienting itself more towards quantitative cell biology and systems biology. To celebrate this, the journal offers us drinks at the Welcome Mixer.

Europe already has a number of Centers for Systems Biology. Of these, the **BioCentrum Amsterdam**, the **Deutsche Krebsforschungszentrum**, the **European Molecular Biology Laboratory, and the European Media Laboratory**, support this course financially. We expect that many Systems Biologist of the future will have been nurtured at these institutions. Likewise Japan has very important institutes, one of which has been world-leading for systems biology, including activities in California. We are pleased that **The Systems Biology Institute** is generously sponsoring this course.

Of course, the home institutions of the organizers have contributed rather importantly to the organization, *i.e.* the **BioCentrum Amsterdam**, the **DKFZ** and the **Max F. Perutz Laboratories of the Vienna Biocenter**. We also thank the **Institute of Molecular Pathology** (IMP) and the **Vienna Veterinary University** for providing poster walls, and the **Institute for Molecular Cell Biology, Amsterdam** for lending some of the additional equipment. Likewise the **Teachers** of the course (*i.e.* the **Lecturers** and the members of the **Scientific Advisory Board: Drs. Aebershold**, **Alberghina**, **Alon**, **Boone**, **Cascante**, **Doyle**, **Eichelbaum**, **Goldbeter**, **Goryanin**, **Heinrich**, **Hohmann**, **Kell**, **Kholodenko**, **Kitano**, **Klingmüller**, **Klipp**, **Kummer**, **LeNovere**, **Noble**, **Reuss**, **Sauer**, **Schuster**, **Snoep**, **Stelling**, **Tomita**, **Van Driel**, **Wanner**, and **Wodak**) have spent quite some time in order to optimize their teaching at this course; their institutes have thereby also contributed.

A course is a matter of human beings, much more than of institutions. This course is possible thanks to the enthusiasm of the many people involved in the actual organization. **Jacky L. Snoep** has provided us with much of the artwork for the abstract book. We thank **Maria Bausback** for secretarial assistance during the course, **Walter Glaser** for helping with the adaptation of the web page, and **Hannes Davidek** of helping with graphic design. Of course the local organizing committee is quite important: we thank **Karen van Eunen**, **Frank Bruggeman**, **Richard Notebaart**, and all others for their contribution to the dynamics of the course. The director and staff of the **Sport und Erlebnis Hotel Gosau** are thanked for the professional way they arrange for infrastructure and food. And we thank **Emilia**, for her patience.

But of course, we should not forget the all-but-silent majority, *i.e.* the participants and their supporters (institutions and mentors), who contributed much effort and inspiration. Reading the abstracts we found that a great many innovative ideas were going to be contributed by the participants *in spe*. This course was the

first of its kind in Systems Biology. Because of the novelty of the field we had applied to FEBS for a course of 120 students. When the number of registrants exceeded 200, we were pleased because it demonstrated great interest and enthusiasm, but saddened because we had to deny many high quality applicants participation. Because quality and potential of most abstracts was high, we also had to select on the basis of more technical parameters, *e.g.* we limited the number of students coming from any same institution. We hope that the students we could not admit will come to a next course. Likewise, we have to admit that although our speakers/teachers are excellent Systems Biologists, we have not been able to attract all excellent Systems Biologist to the course: we had too few speaker slots.

What is next? An exciting course here in Gosau with lots of excellent teaching. The teaching program is special in that it hosts a number of unconventional teaching elements. The latter include the systematic discussion of ***each*** poster contribution by a number of senior scientists, black-board teaching, power-poster presentations, discussion sessions formulating key questions and subsequent sessions trying to address them. Equally importantly, new and more established Systems Biologists from various science directions will meet and discuss science intensively. We expect that Gosau will be the cradle of a network of excellent Systems Biologists who will know to find each other in the future for advice and collaboration. Thus, the interdisciplinary activities that are so important for Systems Biology, take off and make excellent new Science.

The organizers,

and
her *troika* (*i.e.*, Roland Eils , Karl Kuchler , and Hans V. Westerhoff)

Scientific Program - its principles

Symposia

The course has been organized in terms of **4 Symposia,** dedicated to areas within Systems Biology, *i.e.* '**P**rinciples', **T**ools and Methodology', **U**nicellular Organisms and (Cells from) **M**ulticellular Organisms. Each symposium has its dedicated day. On that day the symposium is kicked off with a number of plenary lectures in the morning. Discussions, Workshop talks by invited Principal Investigators, Short talks by invited poster presenters, and a Discussion follow. The posters and power posters corresponding to the symposium are have been grouped together, and will be presented in sequel on the three poster evenings (Sunday, Monday and Wednesday).
Tuesday morning has two extra lectures for symposium T and three extra for symposium U, before the cultural break.

Morning: Plenary Lectures - Discussion of the Issues

Each symposium is kicked off with four plenary lectures in the morning. The lecturer presents an oral presentation for 35 minutes, with a subsequent 10 minutes discussion period. This oral presentation should spend 5 minutes to introduce the field/topic, 15 minutes to teach a few important principles relevant to that topics, and then 15 minutes to report on recent work in which the principles are used in generating some excellent Systems Biology. It is important to realize that it is better to teach little well, than all not at all!
This is followed by a discussion session in which the most cogent Systems Biology questions related to the symposium topic are formulated.

Break for Lunch, Physical Activities, Tea

Lunch will be in the hotel restaurant. Course teachers are requested **not** to seat together. They should rather sit at their own table and be joined by students. Similarly, students are kindly requested **not** to sit together with other students from their own institute, but with Teachers, or with students and principal investigators of other institutes.
After lunch there is a break for physical activities, such as ski-ing, rock climbing, chess, or hang gliding. Be back for tea (coffee if you wish) at 16h00 to engage in the afternoon session that begins at 16h30.

Black-Board Teaching

On Sunday and on Monday, Blackboard teaching sessions will be held after the physical activity break and tea. These are optimized for interactive teaching. Key concepts for Systems Biology will be explained, in interactive mode, using blackboard and chalk, or equivalent. 4 Blackboard presentations will be held in parallel, such that each should be expected to host some 30 students. As a rule, each Blackboard teacher (-couple) presents his Blackboard presentation twice, *i.e.* on Sunday and then again on Monday. Each student is expected to be present at 2 out of 4 Black-board talks.
The following topics have been agreed to:

- Motifs and networks (Alon)
- Stability and flux mode analysis (Heinrich, & Schuster)
- Control analysis and silicon cells (Snoep & Westerhoff)
- Robustness (Stelling & Bruggeman)

Workshop (W) & Short Talks (S)

The topics of workshop presentations and short talks during the afternoon sessions, fall within the area of the main symposium of that particular day (or, in the case of M, of the day thereafter). These talks are usually delivered by principal investigators (W) and students (S), respectively. These speakers have been invited on the basis of their poster abstracts.

Late Afternoon Discussion

During the late-afternoon discussion, the questions raised during the morning discussion session will be addressed by the Lecturers and other Teachers of that symposium. This will be followed by a general discussion. The results of the discussion will be noted down and reported to FEBS, ESF and EUSYSBIO.

Poster Presentations, Poster Committees, Analyses, Discussions

The posters are up throughout the meeting; they should be mounted Sunday evening and removed Thursday evening.
Each poster will be presented for at least an hour by its prime author. Poster numbers *n-2* will be presented/analyzed/discussed Sunday evening from 21h00 for at least an hour. Numbers *3n-1* will be presented/analyzed/discussed Monday evening. Numbers *3n-3* will be presented/analyzed/discussed Wednesday evening. Also the presenters of short talks are requested to present their poster, on the day of their short talk.
Authors presenting posters are asked to indicate on their poster additional times when they will be available at their poster for discussion.
Every student will get to speak the teachers in her/his symposium: each symposium has a corresponding Poster Committee, which consists of all lecturers at that symposium plus:
Symposium **P**rinciples: Alberghina (chair), Westerhoff plus P lecturers
Symposium **T**ools: Cascante (chair), Goryanin plus T lecturers
Symposium **U**nicellular organisms: Hohmann (chair), Kuchler plus U lecturers
Symposium **M**ammalian systems: Van Driel (chair), Kitano plus M lecturers.
During the first 45 minutes of each of the three poster sessions, this committee will inspect the one third of the posters belonging to their symposium that is being presented by their author that evening (*i.e.* up to ten posters). At the end of the poster session, *i.e*. from 22h30 – 23h00), *i.e.* in the 'poster round table discussion', the poster committee will discuss in a session with all poster presenters of their symposium all the posters they have seen that evening (*i.e.* this will be a non-plenary session with approximately 6 committee members and 10 poster presenters).

Power Poster Presentations (PoP's)

Principal investigators who have not been asked to give an oral presentation as Lecture or Workshop Talk, are requested to give a so-called PowerPoster Presentation (PoP). This is a 5 minutes' powerpoint presentation on one of five computers available in the poster halls. They will be asked to run this presentation repeatedly for any PoP viewer interested during the time slot allotted to the PoP presenter. PoP's occur in parallel to the poster presentation by students.

Participant Task List: Contribution/Timing of each Participant

Tasks are AC: Award Committee, B: Black board teaching, C: Chair, L: Lecture, O: Organizer, P: Poster, PC: Poster committee; PoP: Power Poster, S: Short talk plus poster, W: Workshop talk.

Full name	Date	Task	Symposium-Contrib.Number
Ronald Aardema	Sunday	Poster	P-P01
Niels Aarsaether	Sunday	PowerPoster	M-PoP01
Rüdi Äbersold	Monday, S, M, W	Lecture + Poster committee	T-L01 + T-PC
Charles Affourtit	Sunday	Poster	M-P01
Lilia Alberghina	Sunday + S, M,W, Th	Chair + Poster Committee Chair + Award Committee	P-C02 + P-PC + AC
Uri Alon	Wedn + S,M + SMW	Lecture +Blackboard+ Poster Committee	L05 + PT-B1 + U-PC
Ole Herman Ambur	Sunday	Poster	U-P01
Ivan Arisi	Monday	PowerPoster	M-PoP02
Herwig Bachmann	Monday	Poster	U-P02
Stephan Beirer	Monday	Poster	M-P02
Guillaume Beslon	Wednesday	Workshop Talk	U-W01
Martin Bezler	Wednesday	Poster	M-P03
Lars M. Blank	Wednesday	Poster	U-P03
Nils Blüthgen	Thursday	Short Talk + Poster	M-S01
Charlie Boone	Monday + S, M, W	Lecture + Poster committee	T-L04 + T-PC
Irina Borodina	Sunday	Poster	T-P01
Marc Breit	Sunday	Poster	M-P04
Marie Brown	Monday	Poster	P-P02
Frank J. Bruggeman	S, M + S, M + t	Short Talk + Black bpres+Orgr	P-S01, PT-B4
Marina Caldara	Sunday	Poster	U-P04
David Camacho	Wednesday	Poster	P-P03
Marta Cascante	Thurs+ S, M, W + Th	Ch+Post commCh+Aw commCh	M-C02 + M-PC + AC
Cyril Combe	Monday	Poster	T-P02
Holger Conzelmann	Monday	Poster	M-P05
Attila Csikasz-Nagy	Wednesday	Short Talk + Poster	U-S01
R. Keira Curtis	Sunday	Poster	P-P04
Holger Dach	Wednesday	Poster	T-P03
Sune Danø	Monday	Short Talk + Poster	T-S01
Robert P. Davey	Monday	Poster	U-P05
Gianni De Fabritiis	Monday	Poster	P-P05
Alberto de la Fuente	Wednesday	Poster	P-P06
Silvia De Monte	Wednesday	Short Talk + Poster	U-S02
Cathy Derow	Wednesday	Poster	M-P06
Helena Diaz-Cuervo	Sunday	Poster	P-P07
Claudia Donnet	Sunday	Poster	M-P07
Francesco d'Ovidio	Monday	Poster	P-P08
John Doyle	Sunday + S,M,W	Lecture + Poster committee	P-L02, P-PC
Oliver Ebenhöh	Wednesday	Poster	P-P09
Michael Ederer	Sunday	Poster	T-P04
Michel Eichelbaum	Thursday + S, M,W	Lecture + Poster committee	M-L01, M-PC

Thomas Eißing	Monday	Poster	M-P08
Martin Eigel	Wednesday	Poster	M-P09
Roland Eils	Monday + S, M	Lecture + Poster committee	T-L02 + T-PC
Martin Eisenacher	Monday	Poster	T-P05
Graham P. Feeney	Sunday	Poster	M-P010
Raquel Fernandez-Lloris	Monday	Poster	M-P011
Ana Sofia Figueiredo	Wednesday	Short Talk + Poster	U-S03
Emilie S. Fritsch	Wednesday	Poster	T-P06
Tobias Fuhrer	Wednesday	Poster	U-P06
Akira Funahashi	Sunday	Poster	T-P07
Laurent Gaubert	Wednesday	Poster	M-P12
Subhendu Ghosh	Saturday + Sunday	Music + PowerPoster	O-M + T-PoP01
Sergio Giannattasio	Sunday	Poster	U-P07
Adi Gilboa-Geffen	Sunday	Poster	M-P13
Patricio Godoy	Wednesday	PowerPoster	M-PoP03
Albert Goldbeter	Sunday	Lecture + Poster committee	P-L03 + P-PC
Didier Gonze	Monday	Poster	M-P14
Igor Goryanin	Monday + S, M, W,	Chair + Poster committee	T-C02 + T-PC
Niels Grabe	Monday	Poster	T-P08
Reingard Grabherr			
Ioan Grosu	Sunday	PowerPoster	P-PoP01
Vitaly V. Gursky	Sunday	Poster	P-P10
Benjamin A Hall	Wednesday	Poster	T-P09
Kristofer Hallén	Monday	Poster	P-P11
Thomas Handorf	Wednesday	Poster	P-P12
Franz Hartner	Sunday	Poster	T-P10
Mariko Hatakeyama	Thursday	Workshop Talk	M-W01
Feng He	Sunday	Poster	P-P13
Mariela Hebben-Serrano	Monday	Poster	U-P08
Reinhart Heinrich	Sunday	Lect + Blackb pres+Post comm	P-L01 + PT-B2 + P-PC
Julia Heßeler	Monday	Poster	P-P14
Noriko Hiroi	Wednesday	Poster	P-P15
Thomas Höfer	Thursday	Workshop Talk	M-W02
Stephan Hohmann	Tu-,Wedn+S,M,W+Th	Chair+PostCommCh+AwComm	U-C01 + U-PC + AC
Adaoha EC. Ihekwaba	Wednesday	Poster	M-P15
José M. Inácio	Wednesday	Poster	U-P09
Sergii Ivakhno	Monday	Poster	T-P11
Adrienne C. N. James	Monday	Short Talk + Poster	T-S02
Per Harald Jonson	Wednesday	Poster	T-P12
Paula Jouhten	Sunday	Poster	T-P13
Matthieu Jules	Monday	Poster	T-P14
Peter Juvan	Wednesday	Poster	T-P15
Visakan Kadirkamanathan	Monday	PowerPoster	P-PoP02
Douglas B. Kell	Saturday	Opening Lecture	O-L01
Alexander Kern	Sunday	Poster	U-P10
Boris N. Kholodenko	Thursday + S, M, W	Lecture + Poster committee	M-L02 + M-PC
Hiraoki Kitano	Thursday + S, M, W	Chair + Poster committee	M-C01 + M-PC

Ursula Klingmüller	Thursday + S, M, W	Lecture + Poster committee	M-L04 + M-PC
Edda Klipp	Tuesday + S, M, W	Lecture + Poster committee	U-L01, U-PC
Tetsuya J. Kobayashi	Sunday	Poster	P-P16
Markus Kollmann	Sunday	Short Talk + Poster	P-S02
Anneke Koster	throughout	Organizer	O
Konstantin N. Kozlov	Monday	Short Talk + Poster	T-S03
M.T.A. Penia Kresnowati	Monday	Poster	P-P17
Albert Kriegner			
Karl Kuchler	Sun+S, M, W +throug	Chair+Poster Comm +Organizer	T-C01 + T-PC + O
Ursula Kummer	Tuesday + S, M, W	Lecture + Poster committee	T-L02 + T-PC
Lars Küpfer	Monday	Poster	U-P11
Ann Zahle Larsen	Wednesday	Poster	U-P12
Nicolas Le Novere	Thursday + S, M, W	Lecture + Poster committee	M-L03 + M-PC
Dirk Lebiedz	Wednesday	PowerPoster	P-PoP03
Kin Liao	Monday	PowerPoster	T-PoP02
Junli Liu	Sunday	PowerPoster	P-PoP04
Hong-Wu Ma	Wednesday	Poster	P-P18
Shaukat Mahmood	Sunday	Poster	M-P16
Asawin Meechai	Sunday	PowerPoster	U-PoP01
Thomas Millat	Sunday	Poster	P-P19
Liya A. Minasbekyan	Monday	PowerPoster	U-PoP02
Robert Modre-Osprian	Sunday	Poster	T-P16
Hisao Moriya	Monday	Poster	P-P20
Minca Mramor	Monday	Poster	T-P17
Dirk Müller	Sunday	Poster	U-P13
Douglas B. Murray	Wednesday	Short Talk + Poster	U-S04
Leo Neumann			
Ana R. Neves	Monday	Poster	U-P14
Cécile Nicolas	Wednesday	Poster	U-P15
Denis Noble	Thursday	Closing Lecture	O-L02
Richard A. Notebaart	Wednesday +through	Organizer + Poster	T-P18 + O
Jun Ohta	Wednesday	PowerPoster	T-PoP03
Rick Orij	Sunday	Poster	U-P16
Karen Page	Sunday	PowerPoster	M-PoP04
Balázs Papp	Monday	Short Talk + Poster	T-S04
Ainslie B. Parsons	Sunday	Poster	T-P19
Manish Patel	Monday	Poster	T-P20
Mikhail Paveliev	Monday	Poster	M-P17
Venkata G. Peddinti	Wednesday	Poster	P-P21
Esa Pitkänen	Sunday	Short Talk + Poster	P-S03
Jarne Postmus	Monday	Poster	U-P17
Bjørn Quistorff	Wednesday	PowerPoster	T-PoP06
Emma Redon	Wednesday	Poster	U-P18
Matthias Reuss	Tues + W + W, S, M	Lecture+Chair+Poster comm	U-L02 + U-C02 + U-PC
Riccarda Rischatsch	Sunday	Poster	U-P19
Isabel Rocha	Monday	Poster	U-P20
Juan-Carlos Rodriguez	Sunday	Poster	P-P22

Carlos Rodríguez-Caso	Wednesday	Poster	M-P18
Susana Ros	Sunday	Poster	M-P19
Julio Saez-Rodriguez	Monday	Poster	M-P20
Carlos Salazar	Monday	Poster	P-P23
Silvia D. Santos	Thursday	Short Talk + Poster	M-S02
Uwe Sauer	Wednesday	Lecture + Poster committee	U-L04 + U-PC
Thomas Sauter	Thursday	Short Talk + Poster	M-S03
Francesca Maria Scandurra	Wednesday	Poster	M-P21
Jana Schütze	Sunday	Poster	T-P22
Jörg Schaber	Wednesday	Poster	P-P24
H. Schmidt-Glenewinkel	Sunday	Poster	M-P22
Stefan Schuster	Sunday +S,M+S,M,W	Lecture+Blackb+Poster comm	P-L04 + PT-B2 + P-PC
Jacky L. Snoep	Tuesd+ S,M + S,M,W	Lecture+Blackb+Poster comm	T-L05 + PT-B3 + T-PC
Victor Sourjik	Wednesday	Workshop Talk	U-W02
Irena Spasic	Monday	Poster	T-P23
Christian Spieth	Sunday	Poster	P-P25
Dan Staines	Monday	PowerPoster	T-PoP4
Jörg Stelling	Tuesday+S,M+S,M,W	Lecture+Blackbpres+Postcomm	U-L03 + PT-B4 + U-PC
Ara H. Tamrazyan	Wednesday	Poster	U-P21
Sander Tans	Wednesday	PowerPoster	U-PoP3
Bas Teusink	Wednesday	Workshop Talk	U-W03
Rüdiger Thul	Wednesday	Poster	T-P24
Jens Timmer	Monday	PowerPoster	M-PoP5
Masaru Tomita	Wednesday + S,M,W	Lecture + Poster committee	U-L07 + U-PC
Nicolas Tourasse	Sunday	Poster	U-P22
Isil Tuzun	Monday	Poster	U-P23
Renata Usaite	Sunday	Poster	T-P25
Svetlana V. Ustyugova	Monday	Poster	M-P23
Yevhen Vainshtein	Monday	Poster	P-P26
Joost van den Brink	Wednesday	Poster	U-P24
Roel van Driel	Sun, Mon, Wed, Th	Poster Com Chair+Award Com	M–PC+ AC
Frank H.J. van Enckevort	Sunday	Poster	U-P25
Karen van Eunen	Wednesday+through	Poster + Organizer	P-P27 + O
Markku Varjosalo	Wednesday	Poster	M-P24
Vidya R. Velagapudi	Monday	Poster	U-P26
Dennis Vitkup	Sunday	Workshop Talk	P-W01
Todor Vujasinovic	Monday	PowerPoster	P-PoP5
Barry L. Wanner	Wednesday + S,M,W	Lecture + Poster committee	U-L06 + U-PC
Hans V. Westerhoff	Sa,Su,Th + S,M + thr	Chair + Blackboard + Organizer	P-C-1 + PT-B3 + O
Shoshana Wodak	Monday + S, M, W	Lecture + Poster committee	T-L03 + T-PC
Jian Wu	Wednesday	Poster	U-P27
He Yang	Wednesday	PowerPoster	M-PoP6
Sinisa Zampera	Sunday	Workshop Talk	P-W02
An-Ping Zeng	Monday	Workshop Talk	T-W02
Yu Zhang	Monday	Poster	T-P26
Hao Zhu	Sunday	Poster	M-P25
Philip Zimmermann	Wednesday	Poster	T-P27

Course Book - its Principles

Abstracts

All scientists present at the Course have been asked to formulate an abstract of their work or interests in Systems Biology, even those that are too new to the field to have much to report on Systems Biology itself. Most have complied. Accordingly the abstracts vary widely in content and quality. Constructive criticism will be formulated for all student abstracts, and it is in this constructive mode that all discussions should proceed; after all this is a Course, not just a conference. Please note that all abstracts, posters as well as oral presentations, must be considered "*privileged personal communications*". No data may be cited or used in any kind of verbal or written scientific correspondence with third parties without explicit permission of the presenting author.

Course ('Abstract') Book - Paper

The Course book on paper is meant to serve as an in-hand tool at the course. It contains:

- Most Course information
- A list of when each participant has to present her/his work, or fulfill some other function
- The program, described linearly in time, with all presentation represented by their authors and titles
- Abstracts:
    - first the abstracts of the oral presentations in the sequence of the (day-time) program
    - then the abstracts of the poster presentations (including the ones also presented as short talks, and power posters), ordered per Symposium, then per type and then alphabetically.
- List of addresses with presentation code

Abstracts have been giving codes. The first letter refers to the symposium (**P**, **T**, **U**, **M**; for **P**rinciples, **T**ools, **U**nicellular and **M**ulticellular, respectively). The second letter denotes to the type of presentation (L for lecture, W for workshop talk, S for short talk, P for poster, PoP for 'power poster'). Then a sequence number follows. For instance P-P22 refers to poster number 22 in the Symposium on Principles.

- a subject list referring to the abstracts in which the subject is mentioned
- an authors list referring to anywhere where that participant is mentioned in this Course book
- a list of addresses

Course ('Abstract') Book - Electronic

The Course book can also be found as a pdf file on the USB stick provided. The file should be considered non-citable 'preprints'. The program will also be published on the world wide web site (www.FEBSsysbio.net).

Systems Biology Young Investigator Awards

The scientific merit of all abstracts (**posters** and **oral presentations**) submitted by **graduate students** and **postdoctoral researchers** as first authors will be evaluated by the teachers in the corresponding symposium. The best abstracts will be awarded a surprise prize, the **"Gosau Young Investigator Award"** during the Farewell Party. Also two short talk speakers will be awarded such a prize.

Web Site

The course has a website (www.FEBSsysbio.net), which will be *live* before, during and after the meeting. The website can be checked using the wireless network in many areas of the hotel, and using any of the host computers in the poster halls. The abstract book can also be found as a pdf file on the USB stick provided. The poster file should be considered a non-citable 'preprint'. The program will also be published on the world wide web site (www.FEBSsysbio.net).

Technical Local Information

We wish you a very pleasant stay at the venue of the 1st FEBS Advanced Lecture Course on Systems Biology in Gosau. We need to draw your attention to the following points:

Connections – You and the World

The meeting office has a laser printer, a copy machine, as well as phone (+43-6136-8811-390) and FAX (+43-6136-8811-352). Its mobile phone numbers are: +43 676 572 4348 and +43 676 572 434**9**. Any incoming FAX and phone call should clearly identify the addressee. You may not want to use the expensive phone in your hotel room, unless you have a calling card. When available, you can use our phone/FAX machine at regular post office-rates. At the venue, you can be reached, for urgent matters only, at the following e-mail address: hweste@bio.vu.nl, identifying the addressee by having: 'Urgent e-mail for xxx' on the subject line. For non-urgent matters use www.mail2web.com to inspect your own e-mail account, or use www.hotmail.com. At many locations in the hotel there is wireless internet. Computers, some of which are linked to the internet, are available next to the meeting office, as well as in the poster rooms.

Departure

Regular departure from the course is Friday morning after breakfast. At the message board near the Meeting Office there is a 'Departure sheet' which contains your name. Please be so kind to write the date and time of departure you request next to your name. The organizers will 'OK' your name, when they ensured transportation for you to Salzburg airport/train station. Please allow 90 minutes for the transportation from the hotel to the airport (and then of course more than 60 minutes for boarding the flight).

FEBS Evaluation Form

Most importantly, the FEBS EVALUATION FORM!
Please complete and return the lilac FEBS Evaluation Form you will find in your Meeting Pack to the meeting office no later than Thursday, March 17. Any and all criticisms (both positive and negative) are highly appreciated, because we are aware that nothing in this world can be perfect, but many things can be improved. It is imperative that we receive feedback from as many participants as possible (the best of course would be from all of you). Think about it, no return of evaluation forms - no more FEBS Courses on Systems Biology proteins in the future, and, lack of gratitude to FEBS for sponsoring so much of the present course.

FEBS-SysBio2005 Course Office

The meeting office is located in the basement of the Sport & Erlebnis Hotel**** (please follow the signs). If you need help in any way, please contact the meeting office ((+43-6136-8811-390; do not contact the hotel reception desk, please) or call the 24-hour FEBS-SysBio2005 hotline (++43 676 572 4348 and +43 676 572 434**9**). Daily office hours are in the morning from 7.30 – 8.30 am, at noon from 12.00 – 13.00 hours and in the evening from 7.30 – 9.00 pm.

Help

Any member of the local organizing staff, who wear **red** neck cords, will try to help you anytime with any problem you may encounter. Alternatively, turn to the Meeting Office, or call the hotline phone: +43 676 572 4348 and +43 676 572 434**9**

Message Board

Next to the Meeting Office there is a board for messages.

Meals, Beverages & Lunch Packages

Your registration fee includes all meals (breakfast, coffee and tea during the program's tea and coffee breaks, lunch, and dinner) and some non-alcoholic beverages at lunch, dinner and in the poster halls during the poster sessions. Other beverages consumed during lunch and dinner, are not included. For technical reasons, you cannot charge your beverages to your room: You must pay for your beverages at the table in cash in €uros. All beverages and drinks at the Welcome Party (thanks to FEBS Journal) and the Farewell Banquet are free of charge.

If you intend to hit the slopes or otherwise go out early for the afternoon break, you may wish to take a lunch package with you, rather than to eat lunch in the restaurant. You must then pick up a "Lunch Ticket" at the meeting office. Each day has a different color-coded Lunch Ticket with your name on it. You can pick up your Lunch Ticket at the meeting office for any day of the week during regular office hours at the latest, the day before consumption. IMPORTANT, should you for whatever reason not consume your lunch package, you cannot have regular lunch instead on the same day, because the kitchen prepares a limited number of meals, based on the number of meeting participants. Lunch packages themselves can be picked up in the HOTEL BAR around noon time in exchange for YOUR LUNCH TICKET for that day.

Payments

Any substantial payment to the course organization must have been made by giro/bank transfer before the course (cf. www.febssysbio.net). Reimbursements will follow the same route. The course currency is euros. We accept cash (€UR/US$/UK£,JP¥) at current exchange rates (plus exchange cost) we collect from the www (no credit cards). A bank and a cash machine are located on the main road in the nearby village. Banks are open from 8 AM-12 AM and 2 AM to 5 AM in the afternoon (Mon-Fri).

Presentations

Oral presentations: All participants giving oral presentations are requested to be present in the lecture hall half an hour before their session starts (*i.e.* at 8.00 a.m. for talks in the morning and at 16h00 for talks in the afternoon; a member of the organizing committee will assist you). We prefer your files (*i.e.* Powerpoint) as a directory called ''yournameSBcourse' [*e.g.* WesterhoffSBcourse] on a USB stick or CD-ROM. If your presentation links to any other files (*e.g.* movies), these should be in a single directory with the presentation with appropriate links. After copying the directory with your name to the hard disk of either of the two presentation computers in the lecture Hall (*i.e.* a MacIntosh Powerbook and a PC Laptop), you should check whether your presentation and its links actually function. Alternatively, you may connect your own computer to the LCD projector for your talk, but only if you have checked this with the assistant, half an hour in advance.
You can use the computers in the poster halls and near the Meeting Office to check your presentation beforehand.
In case of a presentation that uses media other than LCD projection from computer, please inform the organizers well in advance: hweste@bio.vu.nl.

Posters: Course participants presenting Posters (including presenters of Short Talks) are requested to mount their posters in the dedicated poster areas on the poster board with their poster number (follow the signs) on Saturday evening. Your poster number is identical to the number you will find in the Course book next to the title of your abstract, in the Course book in the address list next to your name, and in the task list in the Course book (*e.g.* P-P04) (a Poster number always has a 'P' for 'Poster', or an 'S' for 'Short Talk' subsequent to the hyphen). Tape and/or pins must NOT be used to mount posters placed behind acrylic glass. If necessary, members of the organizing committee will help you mounting your poster on paper sheets first. For all other poster walls, pins are provided and local organizers will be pleased to assist you if necessary. Posters will stay on display until the evening of Thursday, March 17. The presenting authors needs to be present for at least one hour at the beginning of his poster session. Poster numbers n-2 will be presented / analyzed / discussed Sunday evening from 21h00 for at least an hour. Numbers 3n-1 will be presented / analyzed / discussed Monday evening. Numbers 3n-3 will be presented/analyzed/discussed Wednesday evening. Presenters of short talks are requested to present their poster on the day of their short talk, upstream the posters of their symposium. The dates of presentation can also be gleaned from the *Participant task list* in this course book (*cf.* above).
Power posters ('PopP's): PoP presenters are requested to load a powerpoint file with their presentation onto one of the PC's dedicated to PoP's, which are in the Poster Hall that also houses the PoP's (follow the signs). Numbers 3n-2 will be presented Sunday evening from 21h00 for at least an hour. Numbers 3n-1 will be presented Monday evening. Numbers 3n will be presented Wednesday evening. The dates of presentation can also be gleaned from the *Participant task list* in this Course book (cf. above).
Blackboard presentations: Blackboard Presenters should enquire at the Meeting Office. LCD projector will be available. Presenters are expected to connect their own personal computer. Flipovers will be available as well.
Computer presentations: Anyone whishing to demonstrate a computer program, can do so on an informal basis by making use of the PoP setup, in time slots not allocated to the PoP's.

Skiing and Outdoor Leasure

A daily bus shuttle to the "Hornspitzbahn" organized by FEBSSysBio2005 will leave the hotel 20 minutes after the last morning lecture. The return shuttle from the "Hornspitzbahn" to the Hotel will leave the "Hornspitzbahn" at 4.00 PM sharp. A schedule for the daily public ski bus, as well as a ski route map is included in your registration package. On Saturday and Sunday, you can go to the local ski school, located at the chair lift of the "Hornspitzbahn" for rental equipment such as alpine ski sets, snowboards and cross-country skis. If you show your FEBSSysBio2005 name badge, you will receive a discount on your rental gear. Moreover, you can sign up for skiing lessons, which usually last three to five days. We urge you to finish boot fitting and check-in as soon as possible after your registration, in order to avoid delays during the big rush on Monday.

Social Program

Salzburg. On Tuesday, we have scheduled for all course participants to visit Salzburg, the city of Mozart, with lots of surprises. Buses will leave the hotel at 13h30 and return to the Hotel around 23h30. There will time available for walks or shopping in romantic downtown Salzburg, but there will also be a common program. As you might expect, you should not forget to bring your ears, eyes, and taste buds …… Also be ready to discuss Systems Biology, on the bus, or in the ……..

Depending on interest, we may organize the following excursions (Please enquire at the Meeting Office):

Bad Ischl: A trip to Bad Ischl, the favorite retreat of the one-time Austrian Emperor Franz Josef. Surrounded by a spectacular scenery you can enjoy the rich leisure offered of the magnificent little town Bad Ischl, just like Franz Josef did for more than forty years.

*Hallstad*t: A visit to this restored centre of a Salt and Copper mining town is a thrill.

Ice Cave: A visit to the "Koppenbrüller Ice Cave" leading you into the mighty Dachstein mountain range. Due to expected snowfall, this excursion may not be available.

Sport & Erlebnis Hotel Facilities

The hotel offers an indoor pool, two saunas, steam bath, gym, whirl pool, and solarium at no extra charge to the Course participants. Solarium and whirl pool take tokens that are available free of charge at the hotel reception desk, where further information is also available. Indoor tennis courts are available for a surcharge; please enquire at the hotel reception desk.

Scientific Program of SysBio2005

Saturday March 12

Course Registration & Hotel Check-In **11:00 am - 6:00 pm**

Welcome Reception **6:00 pm - 6:45 pm**

Official Course Opening **6:45 pm - 6:55 pm**

Hans Westerhoff and Karl Kuchler

AstraZeneca Opening Lecture

Douglas Kell **7:00 pm – 8:00 pm**

Metabolomics, machine learning and modelling in systems biology: towards an understanding of the language of cells

Welcome Dinner & Subhendu Ghosh **Musical performance** *Patterns of Passion* **8:30 pm - open end**

Sunday March 13

Breakfast *7:00 - 8:30 am*

Principles of Systems Biology **Lectures** **8:30 am - 12:30 pm**

Chair: Hans Westerhoff

Co-chair: Lilia Alberghina

P-L1 **Reinhart Heinrich** 8:30 - 9:15

Dynamics and design of cellular reaction networks

P-L2 **John Doyle** 9:15 - 10:00

Organizational complexity

Coffee & Refreshment Break *10:00 - 10:20*

P-L3 **Albert Goldbeter** 10:20 -11:55

Computational approaches to cellular rhythms

P-L4 **Stefan Schuster** 11:05 - 11:50

Fundamentals and applications of metabolic pathway analysis

Break *11:50 – 12:00*

Guided General Discussion: **Identifying issues; SB Principles** **12:00 - 12:30 pm**

Lunch & Afternoon Break *12:30 - 4:30 pm*

Coffee and Tea Break *4:00 – 4:30 pm*

Chalk/Blackboard teaching **4 in parallel** **4:30 – 5:10 pm**

PT-B1 Uri Alon — Motifs and networks

PT-B2 Reinhart Heinrich/Stefan Schuster — Stability and flux mode analysis

PT-B3 Jacky Snoep/Hans Westerhoff — Control analysis and Silicon cells

PT-B4 Jörg Stelling/Frank Bruggeman — Robustness, network identification and engineering

Principles of Systems Biology **Workshop & Short Talks** **5:15 – 7:00 pm**

Chair: Lilia Alberghina

Co-chair: Hans Westerhoff

P-W1 **Dennis Vitkup** 5:15 - 5:35

Expression dynamics of a cellular metabolic network

P-S1 **Frank Bruggeman** 5:35 - 5:50

Smart regulation of ammonium assimilation by *Escherichia coli*: modularity, robustness, and flux regulation

Coffee & Refreshment Break *5:50 - 6:10*

P-W2 **Sinisa Zampera** 6:10 -6:30

An adaptive system approach for the modelling of genetic regulatory networks

Glucose metabolism study in the yeast

P-S2 **Markus Kollmann** 6:30 - 6:45

Design principles of signal transduction pathways to attenuate noise

P-S3	**Esa Pitkänen**		6:45- 7:00
	On pathways and distances in metabolic networks		
	Resumed General Discussion:	**Addressing the issues; SB principles**	7:00 - 7:30
Dinner			*7:30 - 9:00 pm*
	Poster Session 1		**9:00 - 11:00 pm**
	Viewing posters		9:00 - 9:45
	Free poster wandering		9:45 – 10:30
	Round table poster discussion (presenters and teachers only)		10:30 – 11:00

Poster Presentations

P-S01 Smart regulation of ammonium assimilation by *Escherichia coli*: modularity, robustness, and flux regulation. ***Frank J. Bruggeman,*** *Fred C. Boogerd and Hans V. Westerhoff*

P-S02 Design Principles of Signal Transduction Pathways to attenuate Noise
Markus Kollmann, *Kilian Bartholome and Jens Timmer*

P-S03 On pathways and distances in metabolic networks
Esa Pitkänen*, Ari Rantanen, Juho Rousu and Esko Ukkonen*

P-P01 The use of accurate mass and time tags to measure yeast's glycolytic proteome
Ronald Aardema, *Henk L. Dekker, Jaap Willem Back, Leo J. de Koning, Luitzen de Jong and Chris G. de Koster*

P-P04 Pathways to analysis of microarray data ***R. Keira Curtis*** *and Antonio Vidal-Puig*

P-P07 A dynamic model of cAMP signal transduction in yeast *Dirk Müller****, Helena Diaz-Cuervo,*** *Luciano Aguilera-Vazquez, Klaus Mauch and Matthias Reuss*

P-P10 Modelling of Drosophila segmentation gene expression with and without usage of attractors
Vitaly V. Gursky, *Johannes Jaeger, Konstantin N. Kozlov, John Reinitz and Alexander M. Samsonov*

P-P13 Inferring gene regulatory relationships from time series microarray data based on the trend of expression changes. ***Feng He*** *and An-Ping Zeng*

P-P16 A reductive approach to analyze stochasticity in intracellular networks.
Tetsuya J. Kobayashi *and Kazuyuki Aihara*

P-P19 Modelling and simulation of dynamic signals in cells. ***Thomas Millat*** *and Olaf Wolkenhauer*

P-P22 An *in silico* model for the optimization of threonine production in *Escherichia coli*.
Juan-Carlos Rodriguez*, Jerome Maury, Christophe Chassagnole, Josep Centelles, Nic Lindley and Marta Cascante*

P-P25 Inferring regulatory networks from experimental data
Christian Spieth*, Felix Streichert, Nora Speer and Andreas Zell*

T-P01 Genome-scale analysis of *Streptomyces coelicolor* A3(2) metabolism
Irina Borodina**,** *Preben Krabben and Jens Nielsen*

T-P04 Reduced order modeling of global regulation - redox regulation in *Escherichia coli*
Michael Ederer*, Thomas Sauter and Ernst Dieter Gilles*

T-P07 CellDesigner2.0: A process diagram editor for gene-regulatory and biochemical networks.
Akira Funahashi*, Naoki Tanimura, Yukiko Matsuoka, Naritoshi Yoshinaga and Hiroaki Kitano*

T-P10 Speeding up the central metabolism in *Pichia pastoris*
Franz Hartner*, Lars Blank, Alexander Kern, Uwe Sauer and Anton Glieder*

T-P13 NMR spectroscopy in systems biology: methods for metabolomics and fluxomics
Paula Jouhten*, Minna Perälä, Eija Rintala Laura Ruohonen, Perttu Permi, Merja Penttilä and Hannu Maaheimo*

T-P16 An integrative framework for modeling signaling pathways ***Robert Modre-Osprian****, Marc Breit, Visvanathan Mahesh, Gernot Enzenberg and Bernhard Tilg*

T-P19 Application of yeast genomic strategies to link biologically active compounds to their cellular targets
Ainslie B. Parsons*, David Williams, Satoru Ishihara, Yoshi Ohya, Raymond Andersen, Timothy Hughes and Charles Boone*

T-P22 Glycolytic oscillations in spatially ordered interacting cells ***Jana Schütze*** *& Reinhart Heinrich*

T-P25 Global transcriptional response of *Saccharomyces cerevisiae* to ammonium, alanine, or glutamine limitation ***Renata Usaite****, Birgitte Regenberg and Jens Nielsen*

U-P01 Neisserial DNA uptake sequences: biased distribution and influence on transformation. ***Ole Herman Ambur**, Stephan Frye, Tonje Davidsen, Hanne Tuven and Tone Tønjum*

U-P04 Experimental manipulation and mathematical modeling of arginine biosynthesis in *Escherichia coli*. ***Marina Caldara**, K. Verbrugghe, L. De Vuyst, M. Crabeel, G. Dupont, A. Goldbeter and R. Cunin*

U-P07 Retrograde response to mitochondrial dysfunction is separable from Tor1/2 regulation of retrograde gene expression. ***Sergio Giannattasio**, Zhengchang Liu and Ronald Butow*

U-P10 Extending life by alternative respiration? ***Alexander Kern**, Franz Hartner and Anton Glieder*

U-P13 A dynamic model of cAMP signal transduction in yeast. ***Dirk Mueller,** Helena Díaz Cuervo, Luciano Aguilera-Vázquez, Klaus Mauch and Matthias Reuss*

U-P16 Stress induced by weak organic acids in *Saccharomyces cerevisiae*. ***Rick Orij,** Jarne Postmus, Gerco van Eikenhorst, Stanley Brul and Gertien Smits*

U-P19 Evolutionary conservation and divergence of fungal promoter sequences ***Riccarda Rischatsch**, Sylvia Voegeli and Peter Philippsen*

U-P22 Unusual group II introns in bacteria of the *Bacillus cereus* group. ***Nicolas Tourasse**, Fredrik Stabell, Lillian Reiter and Anne-Brit Kolstø*

U-P25 LacplantCyc: *in silico* reconstruction of the metabolic pathways of *Lactobacillus plantarum*. ***Frank H.J. van Enckevort**, Bas Teusink, Christof Francke and Roland J. Siezen*

M-P01 Control of the ATP/ADP ratio in pancreatic beta cells ***Charles Affourtit** and Martin D. Brand*

M-P04 Sensitivity analysis with respect to initial values of the TNFalpha mediated NF-kappaB signalling pathway. ***Marc Breit,** Gernot Enzenberg, Visvanthan Mahesh, Robert Modre-Osprian and Bernhard Tilg*

M-P07 Na,K-ATPase regulation via phospholemman phosphorylation ***Claudia Donnet**, Jia Li Guo, Amy Tucker and Kathleen Sweadner*

M-P10 Generating conceptual models in Zebrafish zinc homeostasis: The first steps towards and holistic view of zinc metabolism. ***Graham Feeney**, Dongling Zheng, Peter Kille and Hogstrand Christer*

M-P13 Impaired gene expression in Sjogren's disease. ***Adi Gilboa-Geffen** and Hermona Soreq*

M-P16 Towards a systems biology of signal transduction by insulin and insulin-like growth factors. ***Shaukat Mahmood**, Jane Palsgaard, Soetkin Versteyhe, Maja Jensen and Pierre De Meyts*

M-P19 Molecular dissection of the key LGS residues involved in the control of glycogen biosynthesis. ***Susana Ros** and Joan J. Guinovart*

M-P22 Quantitative modeling of EGFR-internalization as a mechanism of signaling specificity ***Hannah Schmidt-Glenewinkel**, Constantin Kappel and Ivayla Vacheva*

M-P25 Modeling emergent networks by dynamic reconstruction *in silico.* ***Hao Zhu** and Pawan Dhar*

Power Poster Presentations

P-PoP1 New parameter estimation method with possible application in systems biology ***Ioan Grosu***

P-PoP4 Determination of *in vivo* non-steady-state fluxes and kinetic information using stable isotope labeling and metabolite pool size data: theory and application. ***Junli Liu**, Alisdair R. Fernie and David F. Marshall*

T-PoP1 1/f Noise in Ion Channel: A Theory Based on Self-Organised Criticality Jyotirmoy Banerjee, Mahendra K. Verma and **Subhendu Ghosh**

T-PoP4 Using SRS to develop and populate an information layer for the EMI-CD modeling platform ***Dan Staines**, Daniel Flint and Thure Etzold*

U-PoP1 Modeling and analyses of *Mycobacterium tuberculosis* metabolism ***Asawin Meechai**, Supapon Cheevadhanalak and Sakarindr Bhumiratana*

M-PoP1 ***Niels Aarsaether***

M-PoP4 Module dynamics of the GnRH signal transduction network ***Karen Page** and David Krakauer*

Monday March 14

Breakfast *7:00 - 8:30 am*

Tools and methods (part 1) **Lectures** **8:30 am - 12:30 pm**

Chair: Karl Kuchler

Co-chair: Igor Goryanin

T-L1 **Rudi Aebersold** 8:30 - 9:15

Quantitative Proteomics: An essential component of systems biology

T-L2 **Roland Eils** 9:15 - 10:00

Modelling and simulation of large-scale signal transduction networks

Coffee & Refreshment Break *10:00 - 10:20*

T-L3 **Shoshana Wodak** 10:20 - 11:05

Analysing networks of biochemical processes: Bioinformatics meets systems biology

T-L4 **Charlie Boone** 11:05 - 11:50

Global mapping of synthetic genetic interactions in yeast

Break *11:50 – 12:00*

Guided General Discussion: **Identifying issues; Tools, Methods** **12:00 - 12:30**

Lunch & Afternoon Break *12:30 - 4:30 pm*

Coffee and Tea Break *4:00 – 4:30 pm*

Chalk/Blackboard teaching **4 in parallel (repeat)** **4:30 – 5:10 pm**

PT-B1	Uri Alon	Motifs and networks
PT-B2	Reinhart Heinrich/Stefan Schuster	Stability and flux mode analysis
PT-B3	Jacky Snoep/Hans Westerhoff	Control analysis and Silicon cells
PT-B4	Jörg Stelling/Frank Bruggeman	Robustness, network identification and engineering

Tools and methods **Workshop & Short talks** **5:15 - 7:00 pm**

Chair: Igor Goryanin

Co-chair: Karl Kuchler

T-W1 **An-Ping Zeng** 5:15 - 5:35

An integrated interaction network of *Escherichia coli* for studying genotype-phenotype relationship

T-S1 **Sune Danø** 5:35 - 5:50

Oscillatory mechanisms derived from phase and amplitude information

Coffee & Refreshment Break *5:50 - 6:15*

T-S2 **Adrienne James** 6:15 - 6:30

Application of modelling and simulation to drug discovery: The ErbB system

T-S3 **Konstantin Kozlov** 6:30 - 6:45

Combined optimization technique for biological data fitting

T-S4	**Balázs Papp**	6:45- 7:00
	Systematic identification and characterisation of synthetic lethal interactions in the metabolic network of yeast	
	Resumed General Discussion: Addressing the issues Tools & Methods	7:00 - 7:30
Dinner		*7:30 - 9:00 pm*
	Poster Session 2	**9:00 - 11:00 pm**
	Viewing posters	9:00 - 9:45
	Free poster wandering	9:45 – 10:30
	Round table poster discussion (presenters and teachers only)	10:30 – 11:00

Poster Presentations

T-S01 Oscillatory mechanisms derived from phase and amplitude information
***Sune Danø**, Mads Madsen and Preben G. Sørensen*

T-S02 Application of modelling and simulation to drug discovery: The ErbB System
*Bart Hendriks, Gareth Griffiths, Jack Beusmans, **Adrienne James**, Julie Cook, Jonathan Swinton and David De Graaf*

T-S03 Combined optimization technique for biological data fitting
***Konstantin N. Kozlov**, Alexander M. Samsonov and John Reinitz*

T-S04 Systematic identification and characterisation of synthetic lethal interactions in the metabolic network of yeast. ***Balázs Papp**, Richard Harrison, Daniela Delneri, Csaba Pál and Stephen Oliver*

P-P02 Metabolic footprinting: its role in systems biology
***Marie Brown**, Rick Dunn, Julia Handl and Douglas Kell*

P-P05 Multiscale modelling of a cell
***Gianni De Fabritiis** and Peter Coveney*

P-P08 Metabolic quorum sensing: experiments with *S. cerevisiae*
***Francesco d'Ovidio**, Silvia De Monte, Sune Danø and Preben Graae Sørensen*

P-P11 Discovering compound mode of action with CutTree
***Kristofer Hallén**, Johan Björkegren and Jesper Tegnér*

P-P14 Secondary metabolites can create coexistence in the chemostat
***Julia Heßeler**, Julia K. Schmidt, Udo Reichl and Dietrich Flockerzi*

P-P17 Linlog Modeling Approach: Theoretical Platform for System Biology
***M.T.A. Penia Kresnowati**, Wouter van Winden and Sef Heijnen*

P-P20 Systems analysis of yeast glucose sensing system
***Hisao Moriya** and Hiroaki Kitano*

P-P23 Kinetic models of phosphorylation cycles: the role of protein-protein interactions
***Carlos Salazar** and Thomas Höfer*

P-P26 First steps towards a multi-dimensional iron regulatory network
***Yevhen Vainshtein**, Martina Muckenthaler, Alvis Brazma and Matthias W. Hentze*

T-P02 Relational learning of biological networks
***Cyril Combe**, Florence d'Alché-Buc, Vincent Schachter and Stan Matwin*

T-P05 Technical variance, quality control and scaling: necessary steps towards meta-analyses on large expression databases. ***Martin Eisenacher**, Harald Funke, Thomas Vogl, Christoph Cichon, Kristina Riehemann, Clemens Sorg and Wolfgang Koepcke*

T-P08 Simulation of epidermal homeostasis including barrier formation
***Niels Grabe** and Karsten Neuber*

T-P11 Software components for analysis of DNA microarray and quantitative proteomics data
***Sergii Ivakhno** and Olexander Kornelyuk*

T-P14 Autonomous oscillations in *Saccharomyces cerevisiae* during batch cultures on trehalose.
***Matthieu Jules**, Jean-Marie Francois and Jean-Luc Parrou*

T-P17 Data visualization for gene selection and modeling in cancer bioinformatics
***Minca Mramor**, Gregor Leban and Blaž Zupan*

T-P20 SCIpath - an integrated environment for systems biology analysis and visualisation.
Manish Patel

T-P23 Database Support for Yeast Metabolomics Data Management
Irena Spasic, Warwick Dunn and Douglas Kell

T-P26 Identification of the C-terminal signal peptides for GPI modification and prediction of the

cleavage sites. **Yu Zhang**, Thomas Skoet Jensen, Ulrik de Lichtenberg and Soeren Brunak

U-P02 Gene expression and adaptive responses of *in situ* fermentation
***Herwig Bachmann**, Michiel Kleerebezem and Johan E. van Hylckama Vlieg*

U-P05 Comparative metabolomics of Saccharomyces yeasts. ***Robert P. Davey1**, G Lacey1, DA MacKenzie, M Defernez, FA Mellon, K Huber, V Moulton and IN Robert*

U-P08 Unravelling new metabolic metworks in LAB via the thioredoxin system
***L. Mariela Hebben-Serrano**, Eddy Smid and Willem M. de Vos*

U-P11 Systematic computational modelling reveals a key operating principle of TOR signalling in yeast
***Lars Kuepfer**, Matthias Peter, Jörg Stelling and Uwe Sauer*

U-P14 Natural sweetening of food products: engineering *Lactococcus lactis* for glucose production
*Wietske A. Pool, **Ana R. Neves**, Jan Kok, Helena Santos and Oscar P. Kuipers*

U-P17 Adaptation of yeast glycolysis to temperature changes.
***Jarne Postmus**, Jildau Bouwman, Rick Orij, Stanley Brul and Gertien Smits*

U-P20 A Systems Biology approach for the optimization of recombinant protein production in *E. coli*
*Eugénio Ferreira and **Isabel Rocha***

U-P23 The effect of oxygen tension on yeast glycolysis
***Isil Tuzun**, Klaas Hellingwerf and M. J. Teixeira de Mattos*

U-P26 High-throughput screening of *Saccharomyces cerevisae* knockout library: method development and stoichiometric profiling. ***Vidya R. Velagapudi**, Christoph Wittmann, Thomas Lengauer, Priti Talwar and Elmar Heinzle*

M-P02 Regulation of the INF-Gamma/JAK/Stat1 signal transduction pathway
***Stephan Beirer**, Thomas Meyer, Uwe Vinkemeyer and Thomas Höfer*

M-P05 A domain-oriented approach to the reduction of combinatorial complexity in signal transduction networks ***Holger Conzelmann**, Julio Saez-Rodriguez, Thomas Sauter, Boris Kholodenko and Ernst-Dieter Gilles*

M-P08 System Properties of the Core Reactions of Apoptosis
***Thomas Eißing**, Carla Cimatoribus, Frank Allgöwer, Peter Scheurich and Eric Bullinger*

M-P11 Repression of SOX6 transcriptional activity by SUMO modification
***Fernandez-Lloris Raquel**, Osses Nelson, Jaffray Ellis, Shen LinNan, Vaughan Owen Anthony, Girdwood David, Bartrons Ramon, Rosa Jose Luis and Ventura Francesc*

M-P14 Modeling the synchronization of circadian oscillators in the suprachiasmatic nucleus
***Didier Gonze**, Samuel Bernard, Christian Waltermann, Achim Kramer and Hanspeter Herzel*

M-P17 BOOLEAN analysis of the signaling network triggered by neurotrophic factors and extracellular matrix in sensory neurons. ***Mikhail Paveliev**, Maria Lume and Mart Saarma*

M-P20 Analysis of the signaling network involved in the activation of T-Lymphocytes
***Julio Saez-Rodriguez**, Xiaoqian Wang, Birgit Schoeberl, Steffen Klamt, Jonathan Lindquist, Stefanie Kliche, Buckhart Schraven and Ernst Dieter Gilles*

M-P23 Retroelement insertion polymorphism in cell line identification.
***Svetlana V. Ustyugova**, Anna L. Amosova, Yuri B. Lebedev and Eugene D. Sverdlov*

Power Poster Presentations

P-PoP2 Effects of noise in metabolic flux analysis. ***Visakan Kadirkamanathan**, Steve Billings, Sarawan Wongsa, Jing Yang and Philip Wright*

P-PoP5 An adaptive system approach for the modelling of genetic regulatory networks. Glucose metabolism study in the yeast. *Sinisa Zampera and **Todor Vujasinovic***

T-PoP2 Single cell mechanics and mechano signal transduction using a micro-force loading device.
*Hao Zhang, Zhiqing Feng, Ning Fang, Vincent Chan and **Kin Liao***

T-PoP5 Regulatory Network Reconstruction by Integrative Analysis of Cross-Platform Microarray Data.
***Jasmine Zhou**, Ming-Chih Kao, Haiyan Huang, Angela Wong, Juan Nunez-Iglesias, Michael Primig, Oscar Aparicio, Caleb Finch, Todd Morgan and Wing Wong*

U-PoP2 Some changes in the composition of nuclear components during cereal seeds germination.
***Liya A. Minasbekyan** and Poghos H. Vardevanyan*

M-PoP2 SYMBIONIC: A European initiative on the Systems Biology of the neuronal cell ***Ivan Arisi***

M-PoP5 Experimental design for model discrimination in cellular signal transduction
*Clemens Kreutz, Jörg Stelling, Thomas Maiwald and **Jens Timmer***

Tuesday March 15

Breakfast ***7:00 - 8:30 am***

Tools & Methods (part 2) **Lectures** **8:30 am - 10:00 pm**

Chair: Karl Kuchler

T-L5 **Jacky Snoep** 8:30 - 9:15

The Silicon Cell approach to building detailed kinetic models of biological systems

T-L6 **Ursula Kummer** 9:15 - 10:00

Mathematical modelling: Choosing the right simulation method

Coffee & Refreshment Break *10:10 - 10:20*

Unicellular Organisms (part 1) **Lectures** **10:20 am - 12:35 pm**

Chair: Stefan Hohmann

U-L1 **Edda Klipp** 10:20 - 11:05

Mathematical modeling of stress response in yeast

U-L2 **Matthias Reuss** 11:05 - 11:50

Hiding behind the population average - cell cycle dynamics of energy metabolism during the lifelines of individual yeast cells

U-L3 **Jörg Stelling** 11:50 - 12:35

Knowledge and data requirements for systems analysis of cellular networks

Lunch & Afternoon Break *12:35 – 13:15*

VISIT to SALZBURG ***13:30 – 23:00 pm***

Buses will leave Hotel at 13:30 pm

Dinner in Salzburg

Return from Salzburg to the venue 22:00 pm

Wednesday March 16

Breakfast *7:00 - 8:30 am*

Unicellular Organisms (part 2) **Lectures** **8:30 am - 12:30 pm**

Chair: Stefan Hohmann

Co-chair:Matthias Reuss

U-L4 **Uwe Sauer** 8:30 - 9:15

In vivo operation of metabolic pathways

U-L5 **Uri Alon** 9:15 - 10:00

Simplicity in biology

Coffee & Refreshment Break *10:00 - 10:20*

U-L6 **Barry Wanner** 10:20 - 11:05

Stochastic activation of the response regulator PhoB by noncognate histidine kinases

U-L7 **Masaru Tomita** 11:05 - 11:50

Metabolome analysis and systems biology

Break *11:50 – 12:00*

Guided General Discussion: Identifying issues; unicellular organisms 12:00 - 12:30

Lunch & Afternoon Break *12:30 - 4:30 pm*

Coffee and Tea Break *4:00 – 4:30 pm*

Unicellular Organisms **Workshop & Short Talks** **4:30 - 6:50 pm**

Chair: Matthias Reuss

Co-chair: Stefan Hohmann

U-W1 **Guillaume Beslon** 4:30 - 4:50

Modelling evolution of prokaryotic genomes: an integrative approach

U-W2 **Victor Sourjik** 4:50 - 5:10

Signal processing in bacterial chemotaxis

U-W3 **Bas Teusink** 5:10 - 5:30

Combining experimental data and *in silico* analysis to model the metabolic and regulatory network of *Lactobacillus plantarum*

Coffee & Refreshment Break *5:30 - 5:50*

U-S1 **Attila Csikasz-Nagy** 5:50 - 6:05

Modelling fission yeast morphogenesis

U-S2 **Silvia De Monte** 6:05 - 6:20

Metabolic quorum sensing: onset of density-dependent oscillations

U-S3 **Ana Sofia Figueiredo** 6:20- 6:35

Integration of software tools for the *in silico* design of metabolic pathways using flux balance analysis

U-S4	**Douglas Murray**	6:35- 6:50
	Uncovering the control of the respiratory clock in yeast	
	Resumed General Discussion: Addressing the issues; unicellular organisms	6:50- 7:30
Dinner		*7:30 - 9:00 pm*
	Poster Session 3	**9:00 - 11:00 pm**
	Viewing posters	9:00 - 9:45
	Free poster wandering	9:45 – 10:30
	Round table poster discussion (presenters and teachers only)	10:30 – 11:00

Poster Presentations

U-S01 Modelling fission yeast morphogenesis.***Attila Csikasz-Nagy****, Bela Gyorffy, Wolfgang Alt, John J. Tyson and Bela Novak*

U-S02 Metabolic quorum sensing: onset of density-dependent oscillations
Silvia De Monte*, Francesco d'Ovidio, Sune Danø and Preben Grae Sørensen*

U-S03 Integration of software tools for the *in silico* design of metabolic pathways using flux balance analysis. ***Ana Sofia Figueiredo****, Pedro Fernandes, Pedro Pissarra and António Ferreira*

U-S04 Uncovering the control of the respiratory clock in yeast
Douglas B. Murray *and Hiroaki Kitano*

M-S01 Inferring feedback mechanisms in cellular transformation due to oncogenic RAS
Nils Bluethgen*, Christine Sers, Jana Keil, Szymon M. Kielbasa, Reinhold Schaefer and Hanspeter Herzel*

M-S02 Regulation of MAPK signalling determining cell fate in PC-12 cells - a step beyond biochemistry
Silvia D. Santos*, Eli Zamir, Peter Verveer and Philippe Bastiaens*

M-S03 Mathematical modeling of neuronal response to neuropeptides: Angiotensin II signaling via G-protein coupled receptor. ***Thomas Sauter****, Rajanikanth Vadigepalli and James Schwabe*

P-P03 Genetic network model for the AP-1 system. ***David Camacho*** *and Roland Eils*

P-P06 A genetical genomics approach to gene network inference. ***Alberto de la Fuente****, Bing Liu and Ina Hoeschele*

P-P09 Phylogenetic analysis based on structural information of metabolic networks
Oliver Ebenhöh*, Thomas Handorf and Reinhart Heinrich*

P-P12 Scopes: A new concept for the structural analysis of metabolic networks
Thomas Handorf*, Oliver Ebenhöh and Reinhart Heinrich*

P-P15 Two Numerical Model Analyses for the Movement of a Restriction Enzyme.
Noriko Hiroi*, Akira Funahashi and Hiroaki Kitano*

P-P18 Knowledge discovery by integrated analysis of metabolic and regulatory networks
Hong-Wu Ma *and An-Ping Zeng*

P-P21 Investigating the structure of integrated biological networks
Venkata Gopalacharyulu Peddinti*, Erno Lindfors and Matej Oresic*

P-P24 Modelling transient dynamics of osmo-stress response in Yeast.***Jörg Schaber,*** *Bodil Nordlander and Edda Klipp*

P-P27 Nutrient starvation in baker's yeast, and the implication of protein degradation for Vertical Genomics. ***Karen van Eunen****, Jildau Bouwman, Sergio Rossell, Rob J.M. Spanning, Barbara M. Bakker and Hans V. Westerhoff*

T-P03 A new Information System to manage and analyse information on biochemical interactions
Holger Dach*, Juliane Fluck, Kai Kumpf and Rainer Manthey*

T-P06 Genomic rearrangements : influence of the genetic context on chromosomal dynamics
Emilie Fritsch*, Jean-luc Souciet, Serge Potier and Jacky de Montigny*

T-P09 Modelling protein motions for systems biology.***Benjamin A Hall*** *and Mark Sansom*

T-P12 Systemic models for metabolic dynamics and regulation of gene expression – easy access, retrieval and search for publicly available gene expression data.***Per Harald Jonson*** *and M. Minna Laine*

T-P15 Automated construction of genetic networks from mutant data
Peter Juvan*, Gad Shaulsky and Blaz Zupan*

T-P18 Accelerating the construction of genome-scale metabolic models: a test case for *Lactococcus lactis.* ***Richard A. Notebaart****, Frank H.J. van Enckevort, Bas Teusink and Roland J. Siezen*

T-P24 Fokker-Planck equations for IP_3 mediated Calcium dynamics.***Rüdiger Thul*** *and Martin Falcke*

T-P27 The *Genevestigator* gene function discovery engine.***Philip Zimmermann**, Matthias Hirsch-Hoffmann, Lars Hennig and Wilhelm Gruissem*

U-P03 Metabolic functions of duplicate genes in *Saccharomyces cerevisiae*
***Lars M. Blank**, Lars Küpfer and Uwe Sauer*

U-P06 Metabolic network analysis in six microbial species. ***Tobias Fuhrer**, Eliane Fischer and Uwe Sauer*

U-P09 The regulatory circuitry of arabinases in *Bacillus subtilis* .***José M. Inácio** and Isabel de Sá-Nogueira*

U-P12 Dynamic on-line investigation of lactic acid bacteria.
***Ann Zahle Larsen**, Lars Folke Olsen and Frants Roager Lauritsen*

U-P15 Adaptative response of the central metabolism in *Escherichia coli* to quantitative modulations of a single enzyme: glucose-6-phosphate dehydrogenase.***Cécile Nicolas**, Fabien Létisse and Jean-Charles Portais*

U-P18 Progressive adaptation of *Lactococcus lactis* to stress.
***Emma Redon**, Pascal Loubière and Muriel Cocaign-Bousquet*

U-P21 Some properties and partial purification of *Candida Guilliermondii* NP-4 and Paramcium Multimcronucleatum glutaminase. ***Ara H. Tamrazyan**, Misak A. Davtyan and Susanna A. Karapetyan*

U-P24 Vertical genomics in baker's yeast: adaptation of respiring cells to anaerobic sugar-excess conditions. **Joost van den Brink**, Pascale Daran-Lapujade, Han de Winde and Jack Pronk

U-P27 A Sysytems Biology Strategy For Understanding The Genome-wide Control Of Growth Rate And Metabolic Flux In Yeast. *Jian Wu, Nianshu Zhang, Andy Hayes, Douglas Kell, Stephen Oliver and **Jian Wu***

M-P03 Comprehensive analysis of the cancer Tyrosine Kinome & Phosphatome
***Martin Bezler**, Christian Mann, Detlev T. Bartmus, Pjotr Knyazev, Tatjana Knyazeva, Sylvia Streit and Axel Ullrich*

M-P06 Model building in a systems biology company: the cell cycle and apoptosis
***Cathy Derow**, Chris Snell, Christophe Chassagnole, John Savin and David Fell*

M-P09 Meshfree modelling of biological transport processes in complex domains
***Martin Eigel** and Markus Kirkilionis*

M-P12 Network synchronization from population to cell level
***Laurent Gaubert** and Magali Roux-Rouquié*

M-P15 Modelling, Enzyme kinetics & Fluorescence Imaging of the NF-kappaB Signalling Pathway ***Adaoha EC. Ihekwaba**, Rachel Grimley, Neil Benson, David Broomhead and Douglas B. Kell*

M-P18 A topological analysis of the human transcription factor interacting network
***Carlos Rodríguez-Caso**, Miguel Ángel Medina and Ricard V Solé*

M-P21 Flavo-di-iron proteins: role in microbial detoxification by NO
***Francesca Maria Scandurra**, Paolo Sarti, PierLuigi Fiori, Elena Forte, Alessandro Giuffrè, P. Rappelli, G. Sanciu, Daniela Mastronicola, Miguel Teixeira and Maurizio Brunori*

M-P24 RNAi screening for novel components of mammalian Hedgehog and Wnt pathways
***Markku Varjosalo**, Antti Oinas and Jussi Taipale*

Power Poster Presentations

P-PoP3 A new dynamic complexity reduction method for biochemical reaction networks
***Dirk Lebiedz**, Jürgen Zobeley, Julia Kammerer and Ursula Kummer*

T-PoP3 Connectivity matrix for describing all the atom-level connectivities in a given metabolic network and its use for analysis of the network structure.***Jun Ohta***

T-PoP6 Oxygen consumption and glycolytic redox state in skeletal muscle
***Bjørn Quistorff**, Sune Danø, Mads Madsen, Brian Lindegaard Petersen and Peter Fæster Nielsen*

U-PoP3 Differentiation in a genetic network with duplicate repressors: simulating evolutionary pathways based on Lac mutational data. *Frank Poelwijk, Daniel Kiviet and **Sander Tans***

M-PoP3 *In vitro* systems for modelling of signal transduction in hepatocytes
***Patricio Godoy**, Katja Breitkopf, Loredana Ciuclan, Eliza Wiercinska and Steven Dooley*

M-PoP6 Integration of genomics and proteomics with metabolic/signaling pathways for generating/improving novel anti-cancer drug targets. ***He Yang***

Thursday March 17

Breakfast *7:00 - 8:30 am*

Multicellular Organisms **Lectures** **8:30 am - 12:30 pm**

Chair: Hiraoki Kitano

Co-chair: Marta Cascante

M-L1 **Michel Eichelbaum** 8:30 - 9:15

Pharmacogenomics: a holistic approach to drug organism interaction

M-L2 **Boris Kholodenko** 9:15 - 10:00

Systems biology of receptor tyrosine kinase signaling

Coffee & Refreshment Break *10:00 - 10:20*

M-L3 **Nicolas Le Novere** 10:20 - 11:05

Computational systems biology of neuronal signalling

M-L4 **Ursula Klingmüller** 11:05 - 11:50

Signal transduction and cancer – generation of high quality quantitative data

Break *11:50 – 12:00*

Guided General Discussion: Identifying issues; multicellular organisms 12:00 - 12:30

Lunch & Afternoon Break *12:30 - 4:30 pm*

Coffee and Tea Break *4:00 – 4:30 pm*

Multicellular Organisms **Workshop & Short Talks** **4:30 -5:55 pm**

Chair: Marta Cascante

Co-chair: Hiraoki Kitano

M-W1 **Mariko Hatakeyama** 4:30 - 4:50

Computer simulation analysis of ErbB signaling for understanding of cellular transformation mechanism

M-W2 **Thomas Höfer** 4:50 - 5:10

Integration of signal transduction and cytokine expression in T lymphocytes

M-S1 **Nils Bluethgen** 5:10 - 5:25

Inferring feedback mechanisms in cellular transformation due to oncogenic RAS

M-S2 **Silvia Santos** 5:25 - 5:40

Regulation of MAPK signalling determining cell fate in PC-12 cells - a step beyond biochemistry

M-S3 **Thomas Sauter** 5:40- 5:55

Mathematical modeling of neuronal response to neuropeptides: Angiotensin II signaling via G-protein coupled receptor

Coffee & Refreshment Break *5:55 - 6:15*

Resumed General Discussion:Addressing the issues; multicellular organisms 6:15 - 6:45

NovoNordisk Closing Lecture

Denis Noble **7:00 pm – 8:00 pm**

Highlights of SysBio2005: From genes to whole organs

Vertical integration using mathematical simulation

Banquet and Farewell Party **8:00 pm - open end**

Presentation of "Gosau YOUNG SysBio INVESTIGATOR AWARDS" 8:30 - 8:45

Marta Cascante, Lilia Alberghina, Roel van Driel, Stefan Hohmann

Official Course Closure 8:45 - 9:00

Hans Westerhoff and Karl Kuchler

Friday March 18

Breakfast	*7:00 - 8:30 am*
Hotel Check-Out & Departure	**7:00 - 11:00 am**
End of SysBio 2005	11:00 am
Shuttle Buses to Salzburg (detailed schedule to be announced)	

Speaker abstracts

AstraZeneca Opening Lecture

Douglas B. Kell

Metabolomics, machine learning and modelling in systems biology: towards an understanding of the language of cells

School of Chemistry, The University of Manchester, Faraday Building, Sackville St, PO Box 88, MANCHESTER M60 1QD, UK dbk@man.ac.uk http://dbk.ch.umist.ac.uk http://www.mib.ac.uk/ Tel: +44 161 306 4492

Progress in Systems Biology – or in "understanding complex systems" – depends on new technology [1-4], computational assistance [5-7] and new philosophy [8], but probably not in that order (*pace* [9]). Some developments include all three [10, 11]. My lecture will represent an overview encompassing developments and challenges in each of these areas.

1 Kell, D. B. (2004). Metabolomics and systems biology: making sense of the soup. *Curr. Op. Microbiol.* **7,** 296-307.

2 Goodacre, R., Vaidyanathan, S., Dunn, W. B., Harrigan, G. G. & Kell, D. B. (2004). Metabolomics by numbers: acquiring and understanding global metabolite data. *Trends Biotechnol.* **22,** 245-252.

3 O'Hagan, S., Dunn, W. B., Brown, M., Knowles, J. D. & Kell, D. B. (2005). Closed-loop, multiobjective optimisation of analytical instrumentation: gas-chromatography-time-of-flight mass spectrometry of the metabolomes of human serum and of yeast fermentations. *Anal Chem* **77,** 290-303.

4 Kell, D. B., Brown, M., Davey, H. M., Dunn, W. B., Spasic, I. & Oliver, S. G. (2005). Metabolic footprinting and Systems Biology: the medium is the message. *Nat Rev Microbiol***,** submitted.

5 Mendes, P. & Kell, D. B. (1998). Non-linear optimization of biochemical pathways: applications to metabolic engineering and parameter estimation. *Bioinformatics* **14,** 869-883.

6 Ihekwaba, A., Broomhead, D. S., Grimley, R., Benson, N. & Kell, D. B. (2004). Sensitivity analysis of parameters controlling oscillatory signalling in the NF-kB pathway: the roles of IKK and IkBa. *Systems Biology* **1,** 93-103.

7 Brown, M., Dunn, W. B., Ellis, D. I., Goodacre, R., Handl, J., Knowles, J. D., O'Hagan, S., Spasic, I. & Kell, D. B. (2005). A metabolome pipeline: from concept to data to knowledge. *Metabolomics* **1,** 35-46.

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

9 Brenner, S. (1980). *Nature***,** June 5 issue.

10 King, R. D., Whelan, K. E., Jones, F. M., Reiser, P. G. K., Bryant, C. H., Muggleton, S. H., Kell, D. B. & Oliver, S. G. (2004). Functional genomic hypothesis generation and experimentation by a robot scientist. *Nature* **427,** 247-252.

11 Nelson, D. E., Ihekwaba, A. E. C., Elliott, M., Gibney, C. A., Foreman, B. E., Nelson, G., See, V., Horton, C. A., Spiller, D. G., Edwards, S. W., McDowell, H. P., Unitt, J. F., Sullivan, E., Grimley, R., Benson, N., Broomhead, D. S., Kell, D. B. & White, M. R. H. (2004). Oscillations in NF-kB signalling control the dynamics of target gene expression. *Science* **306,** 704-708.

Symposium P

Principles of Systems Biology

Plenary Lectures

P-L01 Dynamics and Design of Cellular Reaction Networks

Reinhart Heinrich

Theoretical Biophysics, Humboldt University, Invalidenstraße 42, Berlin D-10115, Germany, Phone: +49/30/20938698, FAX: +49/30/20938813, e-mail: reinhart.heinrich@biologie.hu-berlin.de, Web: http://www.biologie.hu-berlin.de/~theorybp/

An overview is given about different methods for the mathematical analysis of cellular reaction networks. The lecture focuses on methods of nonlinear dynamics, metabolic control analysis and on methods for elucidating the evolutionary network design. Applications concern glycolysis, kinase/phosphatase cascades and the Wnt-signal transduction pathway. It is shown how bifurcation analysis helps to understand the emergence of complex dynamical behaviour of networks as metabolic oscillations and their synchronization. It is demonstrated how metabolic control analysis (MCA) provides a framework for identifying key components exerting rate limitation in metabolic pathways or playing a crucial role in determining amplitudes and duration of signalling outputs. Calculation of control coefficients is also helpful for quantifying the oncogenic or tumor suppressing effects of proteins, for example in the Wnt-pathway. Moreover, MCA can be used for characterizing the robustness of pathway dynamics against parameter perturbations. As demonstrated for glycolysis special features of the structural design of metabolic networks, such as location of ATP producing and ATP consuming steps, can be explained on the basis of evolutionary optimisation principles. Similar analyses for signal transduction pathways yield the result that kinase/phosphatase cascades should exceed a critical length for transmitting information in fast and reliable way. A new method for elucidating the structural design of metabolic systems is introduced which is based on network expansion starting from certain seed compounds. It allows to draw conclusions concerning the robustness of networks against elimination of reactions as well as concerning the temporal order of the emergence of metabolic pathways during evolution.

P-L02 Organizational complexity

John Doyle

Control and Dynamical Systems, Caltech, 1200 E Cal Blvd, Pasadena CA 91125, US, Phone: 6263954808, FAX: (626) 796-8914, e-mail: doyle@caltech.edu

A surprisingly consistent view on the fundamental nature of complex systems can now be drawn from the convergence of three distinct research themes. First, molecular biology has provided a detailed description of much of the components of biological networks, and the organizational principles of these networks are becoming increasingly apparent. It is now clear that much of the complexity in biology is driven by its regulatory networks, however poorly understood the details remain. Second, advanced technology is creating engineering examples of networks where we do know all the details and that have complexity approaching that of biology. While the components are entirely different, there is striking convergence at the network level of the architecture and the role of protocols, layering, control, and feedback in structuring complex system modularity. Finally, there is a new mathematical framework for the study of complex networks that suggests that this apparent network-level evolutionary convergence both within biology and between biology and technology is not accidental, and follows necessarily from the requirements that both biology and technology be efficient, robust, adaptive, and evolvable. This talk will describe qualitatively in as much detail as time allows these features of biological systems and their parallels in technology, using hopefully familiar and concrete examples. The aim is to be accessible to biologists, and not to depend critically on the mathematical framework. A crucial insight is that both evolution and natural selection or engineering design must produce high robustness to uncertain environments and components in order for systems to persist. Yet this allows and even facilitates severe fragility to novel perturbations, particularly those that exploit the very mechanisms providing robustness, and this “robust yet fragile’” (RYF) feature must be exploited explicitly in any theory that hopes to scale to large systems. If time permits, we will briefly discuss how this view of “organized complexity” contrasts sharply with the view of “emergent complexity” that is favored among researchers who draw their inspiration from models and concepts popular in physics, such as lattices, cellular automata, spin glasses, phase transitions, criticality, chaos, fractals, scale-free networks, self-organization, and so on.

P-L03 Computational approaches to cellular rhythms

Albert Goldbeter

Unité de Chronobiologie théorique, Université Libre de Bruxelles, Campus Plaine, CP 231, Boulevard du Triomphe, Brussels B-1050, Belgium, Phone: +32/2/6505772, FAX: +32/2/6505767, e-mail: agoldbet@ulb.ac.be, Web: http://www.ulb.ac.be/sciences/utc/

Oscillations arise in genetic and metabolic networks as a result of various modes of cellular regulation. In view of the large number of variables involved and of the complexity of the intertwined feedback processes that generate oscillations, computational models and numerical simulations are needed to clarify the molecular mechanism of cellular rhythms. Computational approaches to two major examples of cellular rhythms will be examined. (1) Intercellular communication by pulses of cyclic AMP (cAMP) in Dictyostelium cells provides insights into the function of pulsatile patterns of hormone secretion. Dictyostelium discoideum amoebae aggregate in a wavelike manner after starvation, in response to pulses of cAMP emitted with a periodicity of several minutes by cells behaving as aggregation centers. A model shows that sustained oscillations in cAMP originate from the coupling between a negative feedback loop involving cAMP-induced receptor desensitization and a positive feedback loop due to the activation of cAMP synthesis by extracellular cAMP. The model provides an explanation for the frequency encoding of pulsatile signals of cAMP and for the origin of cAMP oscillations in the course of development. (2) Among biological rhythms those with a circadian (close to 24h) period are conspicuous by their ubiquity and by the key role they play in allowing organisms to adapt to their periodically varying environment. In all organisms studied so far circadian rhythms originate from the negative autoregulation of gene expression. Computational models of increasing complexity will be presented for circadian oscillations in the expression of clock genes in Drosophila and mammals. When incorporating the effect of light, the models account for phase shifting of circadian rhythms by light pulses and for their entrainment by light-dark cycles. Stochastic simulations permit to test the robustness of circadian oscillations with respect to molecular noise. The model for the mammalian circadian clock will be used to address the dynamical bases of physiological disorders of the human sleep-wake cycle. The example of circadian rhythms shows how computational models of genetic regulatory networks can be used to address issues ranging from molecular mechanism to physiological disorders.

P-L04 Fundamentals and Applications of Metabolic Pathway Analysis

Stefan Schuster

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The topological analysis of metabolic networks has attracted increasing interest in recent years. Dynamic mathematical modelling of large-scale metabolic and regulatory networks meets difficulties as the necessary mechanistic detail is rarely available. In contrast, structure-oriented methods such as metabolic pathway analysis only require network topology. In my talk, several concepts central to this analysis are explained: basis vectors of the null-space, enzyme subsets, elementary flux modes [1,2] and extreme pathways [3]. It is shown that the concept of elementary modes is well-suited for determining routes enabling maximum yields of bioconversions and properly describes knockouts. Thus, it is well-suited for analysing redundancy and robustness properties of living cells [4]. Another application is the assessment of the impact of enzyme deficiencies in medicine. The advantages of elementary modes in comparison to basis vectors of the null-space are outlined. The analysis is illustrated by several biochemical examples, such as lysine synthesis in Escherichia coli.
Metabolic pathway analysis for large, complex metabolic networks often meets the problem of combinatorial explosion. One method for coping with this problem is to set all intermediates that participate in more than a threshold number of reactions to external status [5]. Another method is to vary the status of metabolites in such a way that the number of elementary modes is minimized. By both methods, networks can be decomposed into subnetworks.

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Symposium P

Guided General Discussion: **Identifying** issues concerning Systems Biology Principles

Chalk/Blackboard teaching

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Symposium P

Principles of Systems Biology

Workshop Talks

&

Short Talks

P-W01 Expression dynamics of a cellular metabolic network.

Dennis Vitkup

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Towards the goal of understanding system properties of the biological networks we investigate the global regulation of gene expression in the Saccharomyces cerevisiae metabolic network. Our results demonstrate the predominance of local gene regulation in the metabolism. The metabolic genes display statistically significant co-expression on distances smaller than the average network distance. Positive gene co-expression decreases monotonically with distance in the metabolic network, while negative co-expression is strongest at intermediate network distances. We find statistically higher co-expression in the linear sections of metabolism compared to branched pathways. While the majority of the traditionally defined metabolic pathways, when perturbed, display significant of co-expression, only fractions of such pathways are actually regulated together. Generally the structure of the metabolic co-expression is different from the traditional pathway boundaries. Simple topological motifs of the network show distinct co-expression patterns highlighting important design principles of the metabolic dynamics.

P-S01 Smart regulation of ammonium assimilation by *Escherichia coli*: modularity, robustness, and flux regulation.

Frank J. Bruggeman

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Regulation of ammonium assimilation in E. coli is governed by two mechanisms: (i) by glutamine synthetase (GS) and glutamate synthase (GOGAT) and (ii) by glutamate dehydrogenase (GDH). The former system is active at low ammonium concentrations and the latter system gradually takes over ammonium assimilation as function of an increase in the ammonium supply. The net ammonium assimilation flux (J_n) is the sum of both mechanisms. A kinetic model of ammonium assimilation shall be introduced. It will be analyzed in terms of: (i) the robustness of Jn and (ii) the regulation of the individual ammonium-assimilation fluxes of GS/GOGAT (J_{gs}) and GDH (J_{gdh}). The system will be dissected into mechanisms that guarantee nontrivial robustness of J_n by 'smart' regulation of J_{gs} and J_{gdh}. The regulation of Jgs shall be further analyzed in terms of the contributions of different processes. Both types of analysis was carried out in terms of a modular description of the network to facilitate understanding of this complicated regulatory network, which involves feedback regulation, covalent modification, parallel pathways, intracellular signalling via the two-component mechanism and gene expression.

P-W02 An adaptive system approach for the modelling of genetic regulatory networks. Glucose metabolism study in the yeast.

Todor Vujasinovic and **Sinisa Zampera**

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We have used a dynamic neural network to model the yeast glucose metabolism response to glucose deprivation in the culture medium.
Our aim was to produce a predictive rather than explicative model, in order to address the question: 'which molecule of the network should we act upon to obtain a given biological response?' The network was built from literature analysis and KEGG data and includes 133 molecules (3 metabolites, 99 enzymes, 26 transcription factors, 5 signal transduction proteins, connected through 516 interactions). The model was trained by DNA microarray data describing the gene expression response to the fermentation to respiration switch (De Risi et al., Science (1997)278:680-6). The simulation provides a hierarchy of the molecules classified in terms of relative distance to the biological response to be obtained. The model has been applied to the prediction of a gene knock-out response and the detection of the invalidated gene was within acceptable error margins. We will present our model and results and more specifically discuss the redundancy of biological regulatory mechanisms as arguing towards the use of adaptive models, and the impact of the network heterogeneity (scale-free structure) on the learning procedure and inferred parameters.

P-S02 Design Principles of Signal Transduction Pathways to attenuate Noise

Markus Kollmann, Kilian Bartholome and Jens Timmer

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One of the great paradoxes in studying signal transduction pathways is their seemingly oversized topology. Even in rather small signalling cascades like MAP kinase it is unclear why so many kinase reactions are involved and what benefits multi-phosphorylation sites. Similarly one can show in bacterial chemotaxis that the topology can be much more simplified to arrive at almost perfect adaption. These facts give the impression that signalling pathways are rather 'tinkered' than 'properly engineered' [1]. But the underlying assumption within this view on signalling pathways is the concept of 'modularisation' on one hand and moderate component tolerances on the other hand. Only these assumptions allow us to investigate signalling networks ignoring strong intra-cellular perturbations. In this work we show that the topology for bacterial chemotaxis depends crucial on strength of intra-cellular perturbations. We show that chemosensory pathways are not only designed to transmit changes in ligand concentration to the flagella motor proteins under the condition of almost perfect adaption but also to resist inter-cellular noise. For the bacterium *E.coli* the magnitude of variations in concentration of signalling proteins has been measured in detail [2,3] and can vary up to ten-fold between individuals [2]. From the known strength of fluctuations we can interfere the requirements on the topology to attenuate these variations. Under realistic assumptions of variations in binding constants and stochastic noise effects we show that the topology of chemotaxis pathways are not 'tinkered' but the outcome of an evolutionary optimisation process.
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[2] Li M. & G. Hazelbauer, (2004), J.Bact., 186
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P-S03 On pathways and distances in metabolic networks

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Recent 'small-world' studies of the global structure of metabolic networks have been based on the shortest-path distance. As this distance does not capture accurately the complexity of the underlying biochemical processes, we propose new distance measures that are based on the structure of feasible metabolic pathways between metabolites. We define a metabolic pathway as a minimal set of metabolic reactions capable of converting the source metabolites into the target metabolites. The metabolic distance is defined as the number of reactions in a smallest possible pathway connecting the sources to the targets. The production distance is defined as the minimum number of successive reactions needed for such conversion, and is upper-bounded by the first distance. These concepts are defined using an *and-or* graph induced by the metabolic network.
We study the computational complexity and derive algorithms for evaluating the distances. We also provide a linear-time algorithm for finding an upper bound for the metabolic distance which itself is shown NP hard to evaluate. To test our approach in practice, we calculated these and shortest-path distances in two microbial organisms, *S. cerevisiae* and *E. coli*. The results show that metabolite interconversion is significantly more complex than was suggested in previous small-world studies. We also studied the effect of reaction removals (gene knock-outs) on the connectivity of the *S. cerevisiae* network and found out that the network is not particularly robust against such mutations.

Symposium P

Resumed General Discussion: Addressing the issues concerning Systems Biology Principles

Reinhart Heinrich
John Doyle
Albert Goldbeter
Stefan Schuster:

Symposium 

Tools and Methods (Part 1)

Plenary Lectures

T-L01 Quantitative Proteomics: An Essential Component of Systems Biology

Ruedi Aebersold

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Systems biology is the science of dynamic networks of interacting biomolecules. It is based on the insight that such networks have intrinsic properties determining their structure and function that are not apparent from the analysis of the isolated components that constitute the system and that are critical for an understanding of the function and control of the system as a whole. Systems biology was made possible by the availability of the complete genome sequence of the human and other species and by advances in biology, engineering and computer science that have collectively catalyzed the emergence of technologies for the systematic and quantitative measurement of genomic and proteomic profiles and the integrative analysis of the obtained results.
Most biological networks involve proteins. Proteomics, the systematic analysis of proteins is therefore an important component of systems biology. In this presentation we will discuss conceptual and technical advances in proteomics and their application to the analysis of biological networks. Special emphasis will be placed on techniques for the collection and accurate analysis of quantitative proteomics data at high throughput.

T-L02 Modelling and simulation of large-scale signal transduction networks

M. Bentele, H. Busch, I. Vacheva, **R. Eils ***

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Mathematical modeling is required for understanding the complex behavior of large signal transduction networks. Previous attempts to model signal transduction pathways were often limited to small systems or based on qualitative data only. We developed a mathematical modeling framework for understanding the complex signaling behavior of programmed cell death (apotosis). Defects in the regulation of apoptosis result in serious diseases such as cancer, autoimmunity and neurodegeneration. During the last decade many of the molecular mechanisms of apoptosis signaling have been examined and elucidated. A systemic understanding of apoptosis is, however, still missing. To address the complexity of apoptotic signaling we subdivided this system into subsystems of different information qualities. A new approach for sensitivity analysis within the mathematical model was key for the identification of critical system parameters and two essential system properties: modularity and robustness. Our model describes the regulation of apoptosis on a systems level and resolves the important question of a threshold mechanism for the regulation of apoptosis.

T-L03 Analysing Networks of Biochemical Processes: Bioinformatics Meets Systems Biology

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The focus of biology has shifted from the investigation of individual genes and proteins, to the study of large complex networks featuring interactions between tens of thousands of molecular and cellular components. Information on these networks is obtained from genome-scale experimental analyses, which yield very large amounts of valuable but noisy data on biological processes that are still poorly understood. Gaining understanding of these processes remains the major goal. However, given the complexity of the underlying systems this cannot be achieved without efficient means for handling this information, -classically the task of Bioinformatics- and without sophisticated computational approaches for interpreting it in terms of biological knowledge - the aim of Systems Biology-. This is hence where Bioinformatics and Systems biology closely overlap.

We will illustrate this overlap here with examples from our own work. We will briefly describe the aMAZE workbench, which stores and handles information on various types of cellular networks using the framework of graph theory. We will also show how a very simple representation of metabolic networks as weighted graphs can go along way towards building pathways from incomplete information and measuring functional distances between genes. Lastly we will illustrate how simple bioinformatics tools can help in analyzing time- and conditions- dependent aspects of the transcriptional regulation of protein complexes in yeast.

T-L04 Global Mapping of Synthetic Genetic Interactions in Yeast

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We are applying synthetic genetic array analysis to the large-scale mapping of genetic interaction networks in yeast. A yeast genetic interaction network containing ~1000 genes and ~4000 interactions was mapped by crossing mutations in 132 different query genes into a set of ~5000 viable gene deletion mutants and scoring the resultant double mutant progeny for a fitness defect. The average query gene showed ~30 synthetic genetic interactions, indicating that the resulting genetic network is complex and may contain as many as ~100,000 interactions. Connectivity of a gene in the network is predictive of function because query genes tend to interact with genes of related function. Moreover, cluster analysis revealed that subsets of genes displaying similar patterns of genetic interactions may encode components of the same pathway or complex. To investigate networks of essential genes, we created promoter shut-off alleles for over two-thirds of essential yeast genes and proof-of-principle screens show that these strains are compatible with SGA analysis. To extend SGA analysis to synthetic dosage lethality (SDL) and synthetic dosage suppression (SDS) screens, we constructed a plasmid-based yeast array in which each strain expresses a unique, tagged yeast gene from the inducible GAL promoter. We have completed a comprehensive analysis of yeast genes that cause discernible growth defects when overexpressed, and have applied SGA-based SDL/SDS analysis to a number of query genes. In an application of the genetic network analysis, we showed that clustering chemical-genetic profiles and genetic interaction profiles identifies target pathways or proteins, providing a powerful means of inferring mechanism of drug action.

Symposium T

Guided General Discussion: **Identifying** issues concerning Systems Biology Tools and Methods

Chalk/Blackboard teaching

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Tools and Methods

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T-W01 An Integrated Interaction Network of *Escherichia coli* for Studying Genotype-Phenotype Relationship

An-Ping Zeng, Bharani Kumar and Hongwu Ma

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Most recent theoretical studies have provided us with a first transitory perception of the structure of molecular interaction networks in biological systems. In particular, three non-disjoint molecular interaction networks have been the focus of these studies: the metabolic network, the protein–protein interaction network and the transcriptional regulatory network. However these interaction networks only contain information related to specific components like genes, proteins or metabolites but not all the relevant correlated information. Ultimately a more complete picture requires integrating the data obtained from all of these approaches with modeling efforts at different levels of detail. An impressive body of data is already available on *E. coli*. We focus in this study on the reconstruction and analysis of an integrated biological network which incorporates metabolite protein interactions, transcriptional regulation, protein-protein interactions and signal transduction. Along with these interactions, information related with the newly discovered regulators, such as small RNA regulators are also added. First, we determined the metabolite protein interactions. For this purpose we extracted the proteins expressed by all the regulated genes found in an extended transcriptional regulatory network of *E. coli* which contains more than 1050 transcriptional factors (Ma et al. 2004, Nucleic Acids Res. In print). The number of proteins counts to 1283. Out of these, 556 proteins are involved in enzymatic reactions, resulting in the formation of metabolites as output which couple with the transcription factors thereby controlling the gene expression. Then the instances available for the signal transduction system are also added to the network. Mostly the signal transduction and the metabolite protein interactions act as feedback loops in the network. Finally the protein-protein and metabolic interactions are added. From the obtained integrated network further structure analysis on the network is done in order to investigate the organizational structure, network motif and regulation. A multi-layer hierarchical structure with the feedback loops from the bottom layers to the top layers and interactions among the nodes within the same layer illustrates the main feature of the integrated network. This integrated network provides a solid basis for understanding the relation between genotype and phenotype at systems level and is being used for a better reverse engineering of biological networks from proteomic and microarray data.

T-S01 Oscillatory mechanisms derived from phase and amplitude information

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Due to time-scale separation, a dynamical system close to a bifurcation will evolve according to the universal dynamics of that particular bifurcation. We have exploited this fact to devise a novel approach for determining the oscillatory mechanism for systems close to a supercritical Hopf bifurcation. In essence, the method works by identifying the chemical components of the two dynamical modes associated with the oscillatory dynamics: an activating mode and an inhibitory mode. There is no need for prior knowledge of the network structure, the only information required is measurements of the relative phases and amplitudes of the oscillating substances. Hence, metabolomics and mRNA arrays are ideal sources of data. The feasibility of the method is illustrated by its use for analysis of glycolytic oscillations in yeast cells.

T-S02 Application of modelling and simulation to drug discovery: The ErbB System

Bart Hendriks, Gareth Griffiths, Jack Beusmans, **Adrienne James**, Julie Cook, Jonathan Swinton and David De Graaf

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The implication of the ErbB family in the pathogenesis of various cancers has made it a popular target for the development of targeted anti-cancer therapies. ErbB dimerisation, trafficking, and activation are complex processes, making it difficult to intuit how perturbations, such as drug intervention, will affect the system dynamics. We need computational approaches to keep track of and to quantify this complexity. AstraZeneca, in collaboration with the Lauffenburger lab at Massachusetts Institute of Technology, have developed a computational model implementing commonly accepted principles involved in ErbB signal transduction. The current ErbB model is made up of Ordinary Differential Equations (ODEs) and is based on detailed mechanisms of ErbB receptor interactions and downstream signalling components. It contains ~300 species, ~400 parameters and more than 500 reactions. A major challenge in dealing with models of this size is information management and model visualization. Text mining software is used to capture kinetic constants and models are displayed graphically using TeraNode™ Design Suite. Parameter estimation and sensitivity analysis are being exploited to assist model validation. The model is being used to predict the dynamics of receptor phosphorylation in the context of different cell lines and ligand environments. Recent work in our group has demonstrated that a deficiency in internalisation is sufficient to explain the observed signalling phenotype of the Gefitinib-responsive mutants found in NSCLC. Gefitinib ('Iressa'; ZD1839, AstraZeneca, Wilmington, DE) is an ATP-competitive small molecule inhibitor of ErbB1, approved for use in the treatment of non-small cell lung cancer (NSCLC). About 80% of Gefitinib-responsive tumours in NSCLC carry mutations in ErbB1. This model prediction has been experimentally validated using a Gefitinib-responsive and non-responsive NSCLC cell line. The Gefitinib-responsive cell line is shown to be deficient in the internalisation of two ErbB1 ligands, EGF and TGFa. This work provides a mechanistic basis for the link between the role of ErbB1 in oncogenesis and Gefitinib response through decreased internalisation of ErbB1 and increased signalling to AKT.

T-S03 Combined optimization technique for biological data fitting

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Motivation.Development of the organisms from embryo to the adult is one of the central unsolved problems of biology. We are working on characterization problem of systems biology of development in context of the segment determination gene network of a Drosophila embryo. While gene expression is evaluated at a time resolution of a few minutes and a spatial resolution of one cell (see FlyEx database), the regulatory parameters cannot be determined experimentally, and are to be found as the solution of the inverse problem by minimizing the deviation of the model output from the data. We apply a chemical kinetic model describing the dynamics of the expression patterns of the segmentation genes during the blastoderm stage by means of the system of highly non-linear reaction-diffusion equations (Jaeger, J, et al., (2004), Nature, 430, 368).A random search technique, being extremely computationally intensive, is sometimes the only choice for finding the set of parameters that provides the best fit of model to data. Therefore the main problem is to reduce the complexity of finding the parameters of mathematical models.
Results. We developed the Combined Optimization Technique (COT) to reduce the computational cost of solution of the inverse problem of modelling. COT combines advantages of random search and gradient descent. Starting from an arbitrary initial set of parameters, a rough approximation of a minimum is found by the random search, namely, Simulated Annealing (SA), while the final solution is given by Optimal Steepest Descent Algorithm (OSDA), developed earlier (Kozlov, K, et al., (2003), Techn. Physics, 48, 6), and successfully applied as the local optimizer in(Gursky, V, et al., (2004), Phys. D, 197, 286). The dependence of COT convergence of the initial approximation and quality criterion is investigated and the strategy of transition from SA to OSDA is studied here. COT demonstrated high accuracy in reconstruction of model parameters and the 30% total performance benefit in a two-gene network. Further study is performed currently to increase the speed up by application of new automated tuning methods for the OSDA part of COT. *Acknowledgments. The support of the study by the NIH Grants RR07801, TW01147, the CRDF GAP Awards RBO685, RBO1286 is gratefully acknowledged.*

T-S04 Systematic identification and characterisation of synthetic lethal interactions in the metabolic network of yeast

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To what extent and why do the effects of mutations depend on the genetic background? Do deleterious mutations act synergistically? What is the mechanistic basis of genetic interactions and how does it depend on the environment? Answers to these questions are relevant not only to functional genomics, but also to problems such as the evolution of sexual reproduction and how deleterious mutations are eliminated from the population.

Owing to the huge number of potential gene combinations, progress in answering these questions is, however, limited by the lack of efficient genome-scale experimental mapping of genetic interactions. To overcome this difficulty, we propose a combination of *in silico* and *in vivo* studies to screen for synthetic lethal relationships in the yeast metabolic network.

First, we apply flux balance analysis (FBA) to the genome-scale metabolic model of *S. cerevisiae* (Forster et al. 2003) to search for candidate gene pairs showing synthetic lethal interactions. Next, we use laboratory experiments to validate the model's predictions. Our preliminary results suggest that i) FBA is able to predict synthetic lethal interactions, ii) many of the interactions are environment specific, iii) although the density of interactions do not differ significantly between nutrient poor and nutrient rich growth conditions, we observe twice as many genes participating in synthetic lethal interactions in nutrient poor environment and iv) only about 20% of synthetic lethal gene pairs can be explained by the presence of gene duplicates (isoenzymes), this fraction, however, is significantly higher than the 2% previously reported for non-metabolic genes (Tong et al. 2004). The implications of these findings for genetic robustness and phenotypic plasticity are also discussed.

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Symposium 

Resumed General Discussion: **Addressing** the issues concerning Systems Biology Tools and Methods

Rudi Aebersold
Charlie Boone
Roland Eils
Ursula Kummer
Jacky Snoep
Shoshana Wodak

Symposium 

Tools and Methods (Part 2)

Plenary Lectures

T-L05 The Silicon Cell approach to building detailed kinetic models of biological systems

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With the rapid developments in the “omics” fields, the level of detail at which individual cellular components (e.g. mRNAs, enzymes, metabolites) can be described has increased dramatically. Although most of this information is qualitative and often far from complete, for a number of systems, notably metabolic- and signal transduction-pathways, quantitative kinetic information is available to build detailed computer models.
We foresee that such detailed kinetic models will become available for a growing number of (parts of) cellular systems and here propose an approach for the construction of kinetic models such that they can be merged. Such combined models could ultimately describe a complete cell.
The Silicon Cell approach (http://www.siliconcell.net) emphasizes to use kinetic parameter values that are determined experimentally for each of the isolated reaction steps in the system. A clear distinction is made between model construction (on the basis of the characteristics of the isolated components) and model validation (measurements on the complete system). Validated models are collected in a model repository such as JWS Online (http://jjj.biochem.sun.ac.za; http://jjj.bio.vu.nl), where they can be interrogated using a web browser and from where they can be downloaded in standardized format (i.e. SBML). We will illustrate the approach using an existing detailed model for yeast glycolysis (Teusink et al., 2000) and introduce basic concepts of enzyme kinetics and metabolic control analysis.

Teusink B, Passarge J, Reijenga CA, Esgalhado E, Van der Weijden CC, Schepper M, Walsh MC, Bakker BM, Van Dam K, Westerhoff HV, Snoep JL (2000) Can yeast glycolysis be understood in terms of in vitro kinetics of the constituent enzymes? Testing biochemistry. Eur J Biochem 267: 5313-5329

T-L06 Mathematical modelling: Choosing the right simulation method

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The mathematical representation of biochemical systems is a central theme in systems biology. Different methods for the modelling and simulation in this context have been developed so far. These methods are shortly reviewed in this talk. Often, the choice of the method is crucial for the success of the scientific investigation. However, this choice is often done rather arbitrarily or using some heuristics. Given the increasing demand for the usage of mathematical tools even by biologists which might not be expert mathematicians, more rational criteria are necessary to avoid misleading results. Two of the most common simulation methods are the numerical integration of ordinary differential equations (ODEs) and stochastic simulations using *e.g.* the Gillespie algorithm. We have investigated the correlation between the divergence of biochemical systems with the need to employ stochastic methodology. Our results show that calculating the divergence of the system is one possible way to find a rational basis for the decision between different simulation methods.

Symposium U

Unicellular Organisms (part 1)

Plenary Lectures

U-L01 Mathematical modeling of stress response in yeast

Edda Klipp

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The investigation of biological systems has accelerated due to joined efforts in experimental investigation, data analysis and modeling. Yeast is a valuable model system to study the molecular biology, the physiology and the dynamics of cellular stress adaptation. The processes involved in signaling and response and housekeeping constitute a highly interconnected network. The understanding of its performance demands for an integrated investigation of both the subsystems and the network properties based on the knowledge of the pathway structures and experimental data.
Stress response is mediated by signaling pathways. These pathways consist of a set of typical elements: receptors, G proteins, MAP kinase cascades and so on. We will show approaches to model the dynamics of these elements
Mathematical modeling approach will be represented to analyze the response to external signals. The dynamics of the reaction network is described with sets of ordinary differential equations.
The following dynamic aspects of signal transduction will be considered:
Propagation and amplification of the signal: The kinetics of protein-protein interactions determines how fast the signal is transmitted and which level of activation is reached for downstream elements.
Adaptation to stress: The regulatory structure of a pathway decides whether this pathway becomes susceptible or refractory after a first stress. The contribution of different instances of feedback regulation on signal termination will be analyzed.
Integration and separation of signals: Various signal pathways use common elements to transfer different signals. We will investigate the dependence of cross activation on network structure and kinetics.

U-L02 Hiding behind the Population Average - Cell Cycle Dynamics of Energy Metabolism during the Lifelines of Individual Yeast Cells

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A fundamental goal of systems biology is to attain a systems-level understanding of the behaviour of single cells and cell populations. To this end it it of major importance to assess the influence of heterogeneity present in real cell populations on the resulting behaviour observed at the population level; i.e. to what extent does the behaviour of the individual cell during its lifeline differ from the population average which is measured by most assay methods? The differential regulation of energy metabolism during the cell cycle via a cyclic AMP (cAMP)-dependent protein kinase cascade in the yeast Saccharomyces cerevisiae constitutes a characteristic example of this kind. The present work focuses on the central role of the second messenger cyclic AMP (cAMP) in coordinating energy metabolism and cell division via a protein kinase A (PKA)-dependent signaling cascade. Experiments performed in synchronous and continuous yeast cultures have demonstrated distinct cell cycle dynamics of cAMP and its associated regulatory effects of energy metabolism [1]. These results are incorporated into a mathematical model comprising submodules for metabolism (glycolysis and storage carbohydrates), cell growth, cell cycle progression and cAMP signal transduction, most of which exhibit mutual feedback effects. Estimation of model parameters is performed on the basis of own and published experimental data. This guarantees the intimate connection of ex0,periments and model development characteristic of the system biology approach.
The integrated single cell model yields a dynamic description of the cAMP-dependent regulation of metabolism and cell cycle progression during the different cell cycle phases. The chosen modular approach is potentially transferable to systems of medical importance, e.g. when modeling tumor cell behaviour. Moreover, the model can also serve as a basis for a segregated description of heterogeneous cell populations, an issue of major importance in the operation of large-scale bioprocesses.

U-L03 Knowledge and data requirements for systems analysis of cellular networks

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Systems biology aims at understanding complex biological networks through a combination of comprehensive measurements and (quantitative) mathematical modeling. At present, however, it is largely unclear, which knowledge and data will be required for establishing realistic mathematical models. Related to this, it is of equal importance to rigorously ask to what extent the already available data allow for meaningful model development. This talk will address these questions by relying on two examples from metabolic and regulatory network analysis. Steady-state analysis of metabolic networks can start from well-known structural features (e.g. reaction stoichiometries and reversibilities) alone. With this very limited knowledge it is possible to predict network functionalities and control schemes when cellular objectives such as efficiency and robustness are taken into account. In contrast, dynamic is a hallmark of cellular regulation and has to be adequately captured in dynamic mathematical models. Frequently, the associated kinetic parameters are unknown, which could constrain the number of biological systems ‘ready’ for dynamic modeling to very few cases. Systematic analysis for the example of a complex network in yeast cell cycle regulation, however, showed that detailed predictive mathematical models could already be developed based on the known regulatory interactions and a limited set of ’traditional’ experimental data to estimate kinetic parameters. Hence, both examples demonstrate an unexpectedly high degree of information that one can extract from already available biological data and knowledge. More generally, these studies suggest strategies for efficiently linking future experimental and theoretical approaches to cellular networks, and how robustness can help in model development.

*Stelling, J. et al. (2002), Nature **420**: 190.*
*Stelling, J. et al. (2004), Cell **118**: 675.*

Symposium U

Unicellular Organisms (Part 2)

Plenary Lectures

U-L04 *In Vivo* Operation of Metabolic Pathways

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Data on intracellular mRNA, protein, or metabolite concentrations reveal the composition of metabolic networks. In contrast to such compositional information, molecular fluxes through intact metabolic networks link genes and proteins to higher, system-level functions that result from interactions between the components (1). Thus, fluxes are the functional output of the integrated biochemical and genetic interactions within such networks, and are key data for metabolic systems biology. Since such system-level in vivo activities cannot be measured directly, they must be inferred indirectly from measurable quantities. The currently most reliable approach is metabolic flux analysis based on ^{13}C-labeling experiments (2, 3). Recent examples of such network-based intracellular flux quantifications unraveled novel pathways (4) and unexpected reactions (5), thereby questioning the ability of well-known 'textbook' pathways to portray flux through complex metabolic networks. Based on large-scale flux data from ^{13}C-labeling experiments in microtiter plates (6), we investigate the global regulatory structure and functional design principles of bacterial metabolism. In particular, we address the question of whether or not such flux responses to gene deletions can be predicted with any certainty. For this purpose, we use metabolic models of various complexity, whose predictions are compared to the experimental results.

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U-L05 Simplicity in Biology

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Cells are matter that dances. Elaborate structures spontaneously assemble, perform biochemical miracles and vanish effortlessly when their work is done. Moreover, these molecular machines can encode and process information virtually without errors, despite the fact that they are under strong thermal noise and embedded in a dense molecular soup. How could this be? Are there special 'laws of nature' that apply to biological systems that can help us to understand why they are so different from non-living matter?
Recent discoveries suggest that one can, in fact, formulate general laws that apply to biological network design. Since it has evolved to perform functions, biological circuitry is far from random or haphazard. It has a defined style. This is the style of objects that must function, and characterizes both biological and engineered systems. Although evolution works by random tinkering , it converges again and again onto a defined set of circuit elements called network motifs that obey general design-principles.
The goal of this talk is to highlight the design-principles of biological networks. The main message is that biological design contains an inherent simplicity. Although it evolved to function and did not evolve to be understandable to us, simplifying principles may make biological design comprehensible.

U-L06 Stochastic activation of the response regulator PhoB by noncognate histidine kinases

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Two-component systems (TCS) are the most prevalent gene regulatory mechanism in bacteria. A typical TCS is comprised of a histidine kinase (HK) and a partner response regulator (RR). Specific environment signals lead to autophosphorylation of different HKs, which in turn act as phosphoryl donors for autophosphorylation of their partner RRs. Nonpartner HKs and RRs also interact, giving rise to cross regulation among TCSs in response to diverse signals.
PhoR (HK) and PhoB (RR) constitute the TCS for detection of environmental (extracellular) inorganic phosphate (Pi). The PhoR/PhoB TCS controls the expression of a large number of genes for acquisition of alternative phosphorus sources, including phoA, which encodes the non-specific phosphohydrolase bacterial alkaline phosphatase (Bap). Cross activation of PhoB by the nonpartner HK CreC is now a classic example of cross regulation among TCSs. A systematic search for other cross talking HKs revealed five additional HKs that activate (phosphorylate) PhoB (J. M. B. and B. L. W., unpublished data).
Examination of cross activation of PhoB by these non-partner HKs by flow cytometry at the single-cell level revealed a bimodal, “all-or-none,” distribution pattern for expression of a phoAp-gfp (green fluorescent protein) reporter fusion. Although the basis of the observed stochastic behavior is unclear, it seems to reflect an inherent property of TCSs. We propose that cells exploit the stochastic character of TCSs to achieve nongenetic (epigenetic) diversity within genetically homogeneous cell populations in order to facilitate adaptation to environmental changes.

U-L07 Metabolome analysis and systems biology

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Institute for Advanced Biosciences of Keio University has recently developed a novel technology for high-throughput metabolome analysis. The technology is based on capillary electrophoresis electrospray ionization mass spectrometry (CE/MS) and it can simultaneously quantify a large amount of cellular metabolites ranged from 70 to 1,000 molecular weights. Using this technology, we have been conducting a major project on computer modeling of *E.coli*, funded by New Energy and Industrial Technology Development Organization (NEDO) of the Ministry of Economy, Trade and Industry of Japan. The E.coli modeling project has two main goals: (A) to construct a static model of entire metabolic pathways, and (B) to construct a dynamic/simulation model of the primary energy metabolism.
Our approach to constructing entire pathway model consists of three steps: (1) Top down modeling from genomic information, (2) Bottom up modeling from metabolome analysis, and (3) Closing the gap by bioinformatics.
For a dynamic model of primary energy metabolism, we are currently collecting a large amount of metabolome, transcriptome, and proteome data in a systematic manner with various different culture conditions and many different single gene destructive mutants. Those data are then used to construct a computer model using E-Cell System, a software package we have developed for biological simulation.

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Symposium 

Guided General Discussion: **Identifying** issues concerning the Systems Biology of unicellular organisms

Symposium U

Unicellular Organisms

Workshop Talks

&

Short Talks

U-W01 Modelling evolution of prokaryotic genomes: an integrative approach

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Each living system results from an evolutionary process. Thus, understanding the system involves prior understanding of its evolutionary story: How did the complex features we observe *now* emerge? Moreover, living organisms are made up of multiple organisation levels that are all involved in evolution. The phenotype depends on the genotype since DNA encodes the complex protein networks that achieve survival and reproduction functions. But these networks may also influence the genome level. For instance, in bacterial genomes, genes involved in a same biological process form clusters, showing that the metabolic level may indirectly influence the genetic level. However, exploring long term evolution simultaneously on these different organisation levels is obviously impossible. That is why there is a need for *In Silico* models in which virtual organisms evolve during thousands of generations.
With this objective in mind, we have defined the AEVOL model of bacterial genome evolution. In AEVOL, each virtual bacterium competes for reproduction in a virtual environment. Each of them owns a double-strand binary genome, uses it to produce a proteome and expresses a phenotype. The sequence is parsed to detect the virtual genes and a genetic code is used to translate them into proteins. A fuzzy logic framework is used to compute the functional capabilities of the proteins and to combine them in an interaction network in order to compute the organism's global capabilities. All organisms are then compared to the environment and the fittest ones are selected for reproduction. While an organism reproduces, its genome is replicated with eventual random local errors, large scale rearrangements and lateral transfer.
In AEVOL, the lifetime reproductive success of an organism depends on its functional capabilities but not on its genomic structure. However, some specific genome organisations emerge depending on various parameters (environmental features, mutation rates, transfer rates, ...). Thus, AEVOL enables us to explore how functional and genetic organisation level are linked by the evolution (e.g. how gene clusters emerge in bacterial genomes). Our aim is to use AEVOL to investigate how complex biological networks (e.g. gene regulation networks, metabolic networks, ...) appear and are modified during evolution. Indeed, it is well known that the topological properties of complex networks are closely dependent on their history.

U-W02 Signal processing in bacterial chemotaxis

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Chemotaxis in Escherichia coli is one of the most-studied model systems for signal transduction. Receptor-kinase complexes organized in clusters at the cell poles sense chemoeffector stimuli and transmit signals to flagellar motors by phosphorylation of a diffusible response regulator protein. Despite the apparent simplicity of the signal transduction pathway, its high sensitivity, wide dynamic range, and integration of multiple stimuli remain poorly understood. We use an in-vivo assay based on fluorescence resonance energy transfer (FRET) to monitoring in real time changes in the intracellular pathway activity upon chemoeffector stimulation. Using FRET allows a quantitative analysis of signal amplification and integration by the receptor clusters, as well as a quantitative analysis of the adaptation system. In addition, we use fluorescent protein fusions to characterize the cell-to-cell variation (noise) in the expression of chemotaxis proteins. Presentation will compare our experimental data to the predictions made by several recent computer models of chemotaxis.

U-W03 Combining experimental data and in silico analysis to model the metabolic and regulatory network of *Lactobacillus plantarum*

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We have sequenced the complete genome of *Lactobacillus plantarum* WCFS1 (PNAS USA 2003;100:1990). *Lactobacillus plantarum* is a versatile lactic acid bacterium that is important in many food and feed fermentation processes. After prediction of gene function, which is an ongoing process, focus is now on the development and improvement of methods and tools to go from genome sequence to gene annotation, to pathway reconstruction and to prediction of phenotype. Important aspects are how and where to incorporate and use experimental (omics) data, and how and to what extent parts of the process can be automated.
We have set up different bioinformatics tools and experimental techniques in the area of functional genomics. Regulatory networks are being studied by motif searches and promoter/operon predictions, as well as by transcriptome analysis. Tools are in place for visualization of transcriptome data on metabolic maps and on genome-maps (Microbial Genome Viewer: www.cmbi.ru.nl/MGV; encyclopedia of *L. plantarum*: www.lacplantcyc.nl). Although regulated pathways can be identified in this way, it remains difficult to understand the impact of the observed regulation for overall metabolism. For better interpretation and integration of omics data, a genome-scale model of *L. plantarum* was developed. We have reconstructed the metabolic network of *L. plantarum*: Our current network comprises 710 genes (23% of the genome), and 600 reactions. The properties of the metabolic network are being investigated within the framework of constraint-based modeling, and compared with physiological data from continuous fermentations.
In the Netherlands we have set up a consortium for Systems Biology of Lactic Acid Bacteria, in casu *Lactococcus lactis*. Within this consortium we want to use Systems Biology to understand the physico-chemical and biological constraints that limit the growth rate under a set of defined conditions. We are seeking European partners for collaboration in future Systems Biology programs, such as SysMO. For more information, see cmbi4.cmbi.kun.nl/~teusink/SBNL_LAB.

U-S01 Modelling fission yeast morphogenesis

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Because of its regular shape, fission yeast is becoming an increasingly important organism to study cellular morphogenesis. Genetic studies have identified a great number of proteins that are important to regulate shape changes during the cell cycle. Most of these proteins interact with either microtubules or actin, underlining the essential roles these cytoskeletal structures play in cellular morphogenesis. Here we present a simple model for fission yeast morphogenesis that describes the interplay between these cytoskeletal elements. An essential assumption of the model is that actin polymerization is a self-reinforcing process: filamentous-actin promotes its own formation from globular-actin subunits via regulatory molecules. Microtubules stimulate actin polymerization by delivering a component of the autocatalytic actin-assembly feedback loop. We show that the model captures all the characteristic features of polarized growth in fission yeast during normal mitotic cycles. We show that all the major classes of morphogenetic mutants (monoipolar, orb and tea) are natural outcomes of the model. We categorize the types of growth patterns that can exist in our model and compared them with experimental observations.

U-S02 Metabolic quorum sensing: onset of density-dependent oscillations

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Populations of oscillating units coupled by diffusion through a homogeneous medium are studied as a model for cells in a CSTR. In particular, we focus on the dependence of the collective behaviour on the density of the cell suspension. Both the classical Kuramoto model and the recent results on "coupling by quorum sensing" (Garcia-Ojalvo, Elowitz and Strogatz (2004) PNAS 101,10955) indicate that, by diluting the suspension, the cells should keep their oscillatory behaviour while desynchronising.
A different scenario could however take place due to the delay introduced in the coupling by the presence of a medium. In this case, the dilution of the suspension results into the suppression of oscillations at both population and individual levels. Such density-dependent phenomenon may be seen as a metabolic analogous of quorum sensing in bacteria: the amplitudes of the individual metabolic oscillations can provide each individual cell with information on the population density and average state of the population.

U-S03 Integration of software tools for the *in silico* design of metabolic pathways using flux balance analysis

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The systems biology approach, where one can envision the cell as a whole is a step in the direction of narrowing the gap between the rate of data generation and the speed of analysis. This embraces the much desired goal of understanding the role of the metabolic pathways of a determined metabolic network.
This study describes the use of several software tools, integrated to perform the simulation of a specific metabolic pathway by Flux Balance Analysis (FBA). This simulator receives as input the stoichiometry, the thermodynamic and capacity constraints of the metabolic network, and also an objective function. The stoichiometry and the thermodynamic constraints are represented in the SBML format (Systems Biology Markup Language), whereas all the other information is represented in a plain text file. The SBML file is parsed using libSBML, which is a library that can be embedded into an application to read, write and manipulate files in the SBML format. The text file is parsed using FLEX, a lexical analyser that generates a C/C++ program that recognizes specific lexical patterns in the text. With this information, one can construct a Linear Programming (LP) problem. To solve it, GNU lp-solve is used. It uses the simplex algorithm and sparse matrix methods for simple LP problems. The solution provided is a possible flux distribution on the network, that maximises the objective function. In this work, data from a batch fermentation process (where the host system is Escherichia Coli strain BL21) is incorporated in the model definition. In the experiment, Acetate secretion, Oxygen Uptake Rate (OUR), Carbon Evolution Rate (CER) and Biomass production for wild type (wt) and mutant (mt) strains were determined. The mutant was engineered with a plasmid to express the human recombinant interkeukin 4(IL-4) using pRT as a promotor.
The analysis of the flux distribution for wt and mt is performed for the maximisation of ATP production, incorporating as capacity constraints the different data obtained from the experiment. A discrete time analysis was performed, using the same variables, and assuming a steady state for each time sample. Comparing FBA results for wt and mt, the induction of protein in the host system decreases the capacity of producing ATP. The sensitivity of the system to variations in glucose uptake was also performed. It was shown that, in normal conditions, mt and wt were robust to variations with an amplitude of 2% and 20%. When the carbon source is residual, the system shows a higher sensitivity to glucose variations.

U-S04 Uncovering the control of the respiratory clock in yeast

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Continuously growing yeast cultures tend to auto-synchronise producing a robust respiratory oscillation (tau circa 40 min). Recently we have carried out Affymetrix analyses that revealed the majority (>90%) of the transcriptome oscillates within this timeframe [1]. Here we analyse this data using a “Fourier focussing” technique in order to derive transcripts that are closely coupled to the oscillation. The method involved dividing the amplitude calculated by fast Fourier transformation by the mean of the amplitude for three oscillation cycles. This ratio equated to the noise of the transcript’s oscillation; where a perfect sine-wave generates a ratio of one and random data generates a ratio approaching zero. When the ratio was calculated for the yeast transcriptome and plotted, the resulting curve showed two gradients. The intercept of these gradients was used as a noise threshold (ratio of ~0.15; ~1500 genes). The strongly coupled transcripts above this threshold and phenotypic events were then used to construct a “clock face”. A network diagram was then constructed using high quality BIND and transcriptional regulatory networks within Cytoscape [2]. The resulting network consisted of ~1000 transcripts containing the most highly conserved aspects of the eukaryotic process, e.g., ribosome, proteasome, DNA synthesis, autophagy, cyclins, amino acid biosynthesis, carbon metabolism, stress response, respiration, etc. Furthermore two transcriptional sub-graphs out of phase with each other were identified. CIN5, YAP6, YAP1, PHD1 and ROX1 comprised the core of the sub-graph whose transcripts peaked during the low respiratory phase and MET4 and RAD59 comprised the sub-graph whose transcripts peaked during the high respiration phase. The cultures synchronisation mechanism revolves around the production of acetaldehyde and hydrogen sulphide [3], which feed into and out of this network via ALD5/ADH2 and SUL2/MET3 respectively. It is concluded that these networks regulate the respiratory clock within yeast. It is also postulated that this network may form the centre of an energetic “bowtie” common to all eukaryotes because of its high conservation among all eukaryotes.

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Symposium U

Resumed General Discussion: **Addressing** the issues concerning the Systems Biology of unicellular organisms

Uri Alon
Edda Klipp
Matthias Reuss
Uwe Sauer
Jörg Stelling
Masaru Tomita
Barry Wanner

Symposium M

Multicellular Organisms

Plenary Lectures

M-L01 Pharmacogenomics: a holistic approach to drug organism interaction

Michel Eichelbaum

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For all major classes of drugs a substantial proportion of patients will not respond, respond only partially or develop toxicity when standard dose of the particular drug are administered. It has become apparent during the last three decades that mutation of genes encoding for proteins involved in drug disposition and action of drugs can contribute substantially to heterogeneity in the efficacy and toxicity of therapeutic agents.
Drug metabolizing enzymes, in particular cytochrome P450 enzymes play a pivotal role in the elimination process of most drugs. Variability of drug metabolism is responsible for the pronounced interindividual differences in plasma concentrations when patients receive the same dose of a drug. As a consequence variability in drug action and side effects / toxicity ensues. For many phase 1 and phase 2 enzymes catalysing the biotransformation of drugs mutations have been identified. In the case of mutations leading to a loss of function administration of a standard dose of a drug will lead to very high plasma concentrations resulting in exaggerated response, side effects or toxicity. On the other hand gene amplification of enzymes resulting in ultrarapid metabolism of drugs has been identified as a mechanism of poor response. Moreover, in the case of prodrugs which require bioactivation loss of enzyme function is associated with a loss in efficacy therapeutic.
But even if the dose is individualized in order to achieve the same plasma concentrations substantial variability in therapeutic response and side effects will still be observed because concentrations at this site of action can vary substantially. It is increasingly recognized that transfer of drugs in and out of the cells is not a passive process depending on the physiochemical properties, lipophilicity and protein binding but involves active transfer by transport proteins. Because of their localization in intestinal, hepatic and renal epithelial cells, these transport proteins are important for the absorption and elimination of drugs. They play an important role in the targeting drugs to organs because they are localized in blood-organ barriers such as the blood-brain and blood-placenta barrier. Moreover, the same concentration of a drug at the site of action does not necessarily mean identical response because mutations at drug targets (receptors, neurotransmitter transporters, signaling pathways) can profoundly alter the response.

M-L02 Systems biology of receptor tyrosine kinase signaling

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Extracellular information received by plasma membrane receptors, such as G-protein coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), is encoded into complex temporal and spatial patterns of phosphorylation and topological relocation of signaling proteins. Processing and integration of this information through MAPK cascades leads to important cellular decisions ranging from proliferation to growth arrest, differentiation or apoptosis. We applied a combined experimental and computational modeling approach is applied to the EGFR signaling network. We explored kinetic and molecular factors that control the time course of phosphorylation responses, including transient versus sustained temporal activation patterns and oscillations in protein phosphorylation state.
Quantitative analysis of signal transduction is confronted by a combinatorial explosion in the number of feasible molecular species presenting different states of signaling networks that include receptors and scaffold proteins with multiple binding domains. We show that a mechanistic description of a highly combinatorial network generated by various phosphorylation and binding forms of receptors and scaffolds may be drastically reduced using a “domain-oriented” approach, referred to as a macro-model of the network. Modeling of the spatial aspects of GPCR- and RTK-induced signaling emphasizes the importance of receptor-mediated membrane relocation of cytosolic proteins. We demonstrated that the spatial separation of kinases and phosphatases in MAPK cascades may cause precipitous spatial gradients of activated MEK and ERK with high concentration near the cell surface and low in the perinuclear area. The results suggest that there are additional (besides diffusion) mechanisms that facilitate passing of signals from the plasma membrane to distant targets. They may involve endocytosis, scaffolding and active transport of signaling complexes by molecular motors. We hypothesize that ligand-independent waves of receptor activation or/and traveling waves of phosphorylated kinases spread the signals over long distances. In addition to mechanistic modeling, an integrative modular approach to inferring the structure of cellular signaling and gene networks was developed. We demonstrate how dynamic connections leading to a particular module (e.g., an individual gene/protein or a cluster) can be retrieved from experimentally measured network responses to perturbations influencing other modules.

M-L03 Computational systems biology of neuronal signalling

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The latest decade witnessed a drastic modification of our understanding of the signal processing by neurons. It is now clear that signalling pathways are interconnected, and that metabolic and gene regulatory networks should be taken into account. Moreover, the topology of subcellular compartments cannot be ignored any longer, nor considered as frozen. If we want to understand the mechanisms of neuronal signalling, the integration of very heterogenous information is necessary, ranging from kinetic data to geometrical description. That process require large quantity of numerical data. In other term, Neurobiology ought to set its clock to post-genomic time, and move to Systems Biology.

The modeling of neuronal function is a tricky business. First of all, the models have to span several rank orders both in the spatial and temporal dimensions, from the conformational transitions of receptor for neurotransmitters (micro-seconds, nanometers) to the electrical phenomena (seconds, millimeters). This in turn required the use of several different algorithms (stochastic, ordinary and partial equations, cable theory etc.). Secondly, the precise topology of sub-cellular compartments and the location of the biological objects are absolutely crucial for their proper function. A change of the geometry or the density of a patch of receptors will change the post-synaptic potential. The shape of a dendritic spine will strongly affect the impact of this potential on the integrated signal. And molecules such as the calcium calmoduline kinase II translocate between subcompartments as their phosphorylation state is modified. Although some functional modules can be defined, paving the way for distributed simulation, most of the time the portions of program handling the various algorithms have to share memory, in order to continuously update the concentration, location and state of biological objects.

From detailled simulations of a very restricted compartment, such as the post-synaptic densities, to models of the whole cell, Different methods have been used to simulate neuronal function. We shall provide several examples, and discuss the advantages and pitfalls of the various approaches.

M-L04 Signal transduction and cancer – generation of high quality quantitative data

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Cellular responses are controlled by the activation of multiple signaling pathways that form complicated networks of cross-talk and synergisms. Deregulation of the tight control of signal transduction leads to diseases such as the onset of cancer. Although the core components of many signaling pathways have been identified, very little is known how information is processed through these pathways and how cellular decisions are regulated. By combining experimental data with mathematical modeling systems biology opens novel possibilities to analyze and predict the dynamic behavior of signaling pathways deregulated in cancer. However, a major bottle-neck currently is the lack of reliable quantitative data. We developed methods to advance the established techniques of immunoprecipitation and immunoblotting to highly accurate and quantitative procedures. As a consequence, reliable and accurate time-resolved data of phosphorylated and total protein levels can be acquired and absolute concentrations of proteins can be determined. This permits the generation of quantitative data for systems-level analysis and thereby will facilitate the prediction of targets for efficient intervention that could be used for the development of novel anti-cancer therapies.

Symposium M

Guided General Discussion: Identifying issues concerning the Systems Biology of Multicellular organisms

Symposium M

Multicellular Organisms

Workshop Talks

&

Short Talks

M-W01 Computer simulation analysis of ErbB signaling for understanding of cellular transformation mechanism

Kaori Ide [1], Takeshi Nagashima [1], Yoshiki Yamaguchi [1], Takashi Naka [2], Shuhei Kimura [3], Atsushi Suenaga [1], Makoto Taiji [1] and **Mariko Hatakeyama [1]**

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Deregulation of ErbB receptors is implicated in various kinds of human cancers. We made mathematical models of the ligand-induced ErbB signal transduction pathways based on the experimental data using the ErbB receptor expressing CHO cells. The coexpression of the different ErbB receptors induced changes in biochemical dynamics as well as in protein-protein interaction patterns. Interestingly, the coexpression of different ErbBs induced specific biological architectures such as integration of pathways (e.g. activation of B-Raf in addition to Raf1 activation) that enable to amplify an initial signal. And those changes in signaling pathways affect the following gene expression profile.
On the other hand, protein-protein interaction (PPI) dynamics plays an important role to stimulate or attenuate signal transduction pathway and define the later cellular state. We apply molecular dynamics (MD) simulation of signaling proteins, such as Grb2, PI3K and Shc, for understanding of regulatory mechanism of signal transduction pathways. MD simulation well predicted the binding affinities of PPI and binding properties. Such a molecular knowledge can be applied for precise mathematical modeling of signal transduction network.

M-W02 Integration of signal transduction and cytokine expression in T lymphocytes

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In the course of an immune response, cytokines received and expressed by T cells play a pivotal role in regulating their proliferation, effector function, and differentiation into memory cells. We have studied the regulatory networks involved in cytokine signaling at different temporal and spatial scales. Intracellular signal transduction is mediated by cycles of reversible phosphorylation and nuclear transport of Stat transcription factors. By a combination of mathematical modeling and experimentation, we have identified critical control steps of the interferon/Stat1 network. Based on this analysis, we were able to design a Stat1 mutant whose altered kinetic parameters render it a more potent transcriptional activator than the wildtype protein. Thus signaling pathways may not be optimized solely for efficient relay and amplification. Rather, the sensitivity of the Stat1 pathway can be adapted to its input signal. The Stats commonly induce their activating cytokines and cytokine receptors, which results in autocrine feedback loops. A kinetic model for the growth factor interleukin-2 shows how multistationarity caused by autocrine signaling can underlie the discrete decision of a cell on its future proliferation. The system exhibits complex dynamics of intercellular communication which explain seemingly paradoxical findings on the observed functions of interleukin-2 as a T cell activator and a suppressor of immune responses. Interestingly, the feedback regulation of the expression of signal transduction components is a frequent motif in T cell cytokine signaling. We will discuss the implication of this design for developing mechanistic models of gene-regulatory networks.

M-S01 Inferring feedback mechanisms in cellular transformation due to oncogenic RAS

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Intracellular signaling cascades display distinct activation profiles in response to various stimuli. Such activation patterns are strongly influenced and shaped by feedback loops. Different feedback loops can act in a cell context- and stimulus-dependent manner and produce a variety of temporal activation profiles, including oscillations and hysteresis. The MEK-ERK cascade plays an important role in cell-cycle regulation, differentiation and in cell transformation caused by oncogenic RAS. This cascade is regulated by several positive and negative feedback loops and is essential for signal transmission due to many different stimuli. While post-translational feedback loops have been subject to extensive mathematical modeling, feedbacks that are mediated by transcriptional control are still poorly understood.

Using a combination of time-course experiments, mathematical modeling and bioinformatic analysis we investigate the effect of transcriptional feedback regulation in cellular transformation following induction of oncogenic RAS. In fibroblasts harboring an inducible RAS oncogene, we monitor the phosphorylation of ERK1,2 by Western Blot analysis. In addition, we analyze the expression profiles of RAS target genes with microarrays in a time-resolved manner. The phosphorylation of ERK shows a biphasic response upon constant induction and an oscillatory response after brief induction of RAS. We find that several dual specific phosphatases are expressed with similar kinetics. A bioinformatic analysis unveils two ERK-dependent transcription factors that control this battery of phosphatases. Together with the transcription factors, these phosphatases constitute a negative feedback for ERK-activity. Mathematical modeling and experimental interference shows that we can explain the biphasic and oscillatory dynamics as a result of phosphatase activation.

M-S02 REGULATION of MAPK signalling determining cell fate in PC-12 cells - a step beyond biochemistry

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Mitogen activated protein kinase (MAPK) cascades participate in a wide array of cellular transduction programmes including cell growth and division, movement, differentiation and cell death. A paradigm system to study how the activity of these cascades produces different cell responses is the PC-12 cells system. In these cells the classical ERK pathway is activated by both EGF and NGF, giving rise to cellular opposite fates – division and differentiation, respectively. We believe different biochemical topology may be the key determining these specific responses. We are therefore interested in measuring reaction states of main components of this pathway, to analyze how the kinases are spatially organized and biochemically connected. We are using polychromatic fluorescence activating cell sorting (FACS) with phospho-labelled antibodies, which detect the active state of network components. By applying systematic perturbations of activities and subsequent read out on multiple reaction states at steady-state we are able to retrieve information on the network topology. Single cell measurements are being performed and RNAi and pharmacological inhibitors used for the perturbations. Moreover, response coefficients for each kinase, before and after perturbations will be calculated and first order connectivity maps built.
In addition, by using fluorescence resonance energy transfer (FRET) imaging with multiple optical sensors, reaction states of kinases and their spatial information are being determined simultaneously in one cell. Fusion proteins of GFP mutants and pathway kinases allow the detection of protein-protein interactions and molar ratios of phospho-proteins can be detected, by using phospho-antibodies against phospho-residues on active kinases.

M-S03 Mathematical modeling of neuronal response to neuropeptides: Angiotensin II signaling via G-protein coupled receptor

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In neurons G-protein coupled receptors (GPCRs) are involved in the alteration of neuronal activity (neuromodulation) via cascades of interacting proteins. The complex dynamic behavior of these networks, e.g. the integration of different signals, cannot be understood by intuition alone. Mathematical modeling provides an appropriate tool to decipher this complexity. Angiotensin II and AT1 receptor dependent signaling was investigated as an examples that use GPCR signaling pathways (Gq). AT1 signals via a wide variety of intracellular signaling molecules, involving (1) G-protein mediated stimulation of phospholipase C (PLC), with subsequent Ca2+ mobilisation; (2) Jak/STAT pathway; (3) transactivation of tyrosine kinase pathways. Relevant signaling outputs are modified gene expression patterns and modified neuronal activity via changes in membrane ionic currents and firing rate.
New data that was collected recently [Fernandez et al., Hypertension Jan.2003:56-63] showed that Angiotensin II can elicit stimulating and suppressive effects in the same neurons in dependency of the basal Ca2+ level. We have built a detailed mechanistic model of Angiotensin II signaling that captures both the stimulating and suppressive effects. This ODE model includes the AT1 mediated activation of PLC and PKC, and IP3 and channel mediated variation of the cytosolic Ca2+ level after Angiotensin II stimulation (adapted from [Mishra and Bhalla, Biophys. J., 83:1298-1316, 2002]). Based on in silico simulations of this model, we hypothesize that the observed biological variability is based on cell-to-cell variation in the dynamics of the Na-Ca exchanger.
Furthermore, a Hodgkin-Huxley model approach is used to investigate the function of cell signaling in altering the firing behavior of NTS neurons in response to various baroreceptor stimuli. Angiotensin II was found to activate neuronal firing in low firing NTS neurons.
In summary, detailed mathematical is a valuable tool to understand and investigate neuronal response to neuropeptides and furthermore to link signal transduction to the electrophysiological behavior of neurons.

Symposium M

Resumed General Discussion: Addressing the issues

Michel Eichelbaum
Boris Kholodenko
Nicolas Le Novere
Ursula Klingmüller

NovoNordisk Closing Lecture

Highlights of SysBio2005: From genes to whole organs

Vertical integration using mathematical simulation

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Biological modeling of cells, organs and systems has reached a very significant stage of development. Particularly at the cellular level, there has been a long period of iteration between simulation and experiment (Noble 2002a). We have therefore achieved the levels of detail and accuracy that are required for the effective use of models in systems biolocal research and in drug development. To be useful in this way, biological models must reach down to the level of proteins (receptors, transporters, enzymes etc), yet they must also reconstruct functionality right up to the levels of organs and systems (Noble 2002b). I will illustrate these points with reference to both the proceedings of this course and models of the heart (Noble 2002c). The lecture will use this work to illustrate some fundamental principles of Systems Biology.

Noble D (2002a) The Rise of Computational Biology. Nature Reviews Molecular Cell Biology, 3, 460-463

Noble D (2002b) Modelling the heart: insights, failures and progress. Bioessays 24, 1155-1163

Noble D (2002c) Modelling the heart: from genes to cells to the whole organ. Science 295, 1678-1682

Abstracts of Posters

&

Abstracts of Power Posters

Principles

Posters

P-S01 Smart regulation of ammonium assimilation by *Escherichia coli*: modularity, robustness, and flux regulation.

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Regulation of ammonium assimilation in E. coli is governed by two mechanisms: (i) by glutamine synthetase (GS) and glutamate synthase (GOGAT) and (ii) by glutamate dehydrogenase (GDH). The former system is active at low ammonium concentrations and the latter system gradually takes over ammonium assimilation as function of an increase in the ammonium supply. The net ammonium assimilation flux (J_n) is the sum of both mechanisms. A kinetic model of ammonium assimilation shall be introduced. It will be analyzed in terms of: (i) the robustness of J_n and (ii) the regulation of the individual ammonium-assimilation fluxes of GS/GOGAT (J_{gs}) and GDH (J_{gdh}). The system will be dissected into mechanisms that guarantee nontrivial robustness of Jn by 'smart' regulation of Jgs and Jgdh. The regulation of J_{gs} shall be further analyzed in terms of the contributions of different processes. Both types of analysis was carried out in terms of a modular description of the network to facilitate understanding of this complicated regulatory network, which involves feedback regulation, covalent modification, parallel pathways, intracellular signalling via the two-component mechanism and gene expression.

P-S02 Design Principles of Signal Transduction Pathways to attenuate Noise

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One of the great paradoxes in studying signal transduction pathways is their seemingly oversized topology. Even in rather small signalling cascades like MAP kinase it is unclear why so many kinase reactions are involved and what benefits multi-phosphorylation sites. Similarly one can show in bacterial chemotaxis that the topology can be much more simplified to arrive at almost perfect adaption. These facts give the impression that signalling pathways are rather 'tinkered' than 'properly engineered' [1]. But the underlying assumption within this view on signalling pathways is the concept of 'modularisation' on one hand and moderate component tolerances on the other hand. Only these assumptions allow us to investigate signalling networks ignoring strong intra-cellular perturbations. In this work we show that the topology for bacterial chemotaxis depends crucial on strength of intra-cellular perturbations. We show that chemosensory pathways are not only designed to transmit changes in ligand concentration to the flagella motor proteins under the condition of almost perfect adaption but also to resist inter-cellular noise. For the bacterium *E.coli* the magnitude of variations in concentration of signalling proteins has been measured in detail [2,3] and can vary up to ten-fold between individuals [2]. From the known strength of fluctuations we can interfere the requirements on the topology to attenuate these variations. Under realistic assumptions of variations in binding constants and stochastic noise effects we show that the topology of chemotaxis pathways are not 'tinkered' but the outcome of an evolutionary optimisation process.

[1] Alon U., (2003), Science, 301
[2] Li M. & G. Hazelbauer, (2004), J.Bact., 186
[3] Elowitz M. et al., (2002), Science, 297

P-S03 On pathways and distances in metabolic networks

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Recent 'small-world' studies of the global structure of metabolicnetworks have been based on the shortest-path distance. As this distance does not capture accurately the complexity of the underlying biochemical processes, we propose new distance measures that are basedon the structure of feasible metabolic pathways between metabolites. We define a metabolic pathway as a minimal set of metabolic reactions capable of converting the source metabolites into the target metabolites. The metabolic distance is defined as the number of reactions in a smallest possible pathway connecting the sources to the targets. The production distance is defined as the minimum number of successive reactions needed for such conversion, and is upper-bounded by the first distance. These concepts are defined using an and-or graph induced by the metabolic network.
We study the computational complexity and derive algorithms for evaluating the distances. We also provide a linear-time algorithm for finding an upper bound for the metabolic distance which itself is shown NP hard to evaluate. To test our approach in practice, we calculated these and shortest-path distances in two microbial organisms, *S. cerevisiae* and *E. coli*. The results show that metabolite interconversion is significantly more complex than was suggested in previous small-world studies. We also studied the effect of reaction removals (gene knock-outs) on the connectivity of the *S. cerevisiae* network and found out that the network is not particularly robust against such mutations.

P-P01 The use of accurate mass and time tags to measure yeast's glycolytic proteome.

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Within the Vertical Genomics project, focus lies on the glycolysis of *Saccharomyces cerevisiae*. This pathway will be studied with respect to promoter activities, mRNA levels, protein concentrations, enzyme activities, metabolite concentrations and metabolic fluxes, under well defined growth conditions.
The high-throughput proteomics method, presented here is based on a combination of stable ^{15}N isotope labeling of a reference culture to produce isotopically labeled internal standards for calibration purposes and the use of accurate mass and time tags (AMT tags) to quantify $^{14}N/^{15}N$ isotope ratio's of glycolytic peptides obtained by tryptic digestion of whole yeast cell lysates.
Peptides of 14 glycolytic enzymes are readily detected. However, nanoLC-QTOF-MS/MS measurements and data processing with our in-house developed Virtual Mass Spectrometry Lab software package have shown the need to invoke normalized LC retention times of glycolytic peptides. To increase coverage of all enzymes of interest simple strong cation exchange chromatography is used.
Once unique peptides of the proteins of interest are found (using MS/MS), the accurate mass is calculated and stored in a database together with the normalized retention time during reversed phase chromatography. This retention time in combination with high mass accuracy will be used to obtain unique AMT tags. With these validated AMT tags, differences in protein concentration can be measured between the ^{15}N labeled glycolytic subproteome of the reference culture and yeast culture grown under varying experimental conditions with LC-FTICR-MS analysis.
The data will be correlated with data from other hierarchic levels of the pathway such as the transcriptome, enzyme activities and the metabolome to gain insight in higher order regulation of glycolysis.

P-P02 Metabolic footprinting: its role in systems biology

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Of the 6000 genes sequenced in the yeast *Saccharomyces cerevisiae* genome fewer than 50% have function that is confidently known. One approach to determine gene function is the metabolome-based analysis of single gene knockout mutants. Metabolic footprinting is concerned with the analysis carried out where the extra-cellular metabolites secreted into the culture medium are analysed and provides a rapid and non-invasive methodology (Allen et al. 2003). Analytical technologies employed include chromatography-mass spectrometry, direct injection mass spectrometry and Fourier Transform-Infra Red spectroscopy (Dunn et al. 2004).

A variety of methods as detailed below are then used to obtain information about the gene knockouts and determine which show similar patterns of metabolic changes. Generally gene functionality can be determined by calibration of genes of known function with those of unknown function or by comparison of the metabolic profiles of mutant with wild-type strains.

• standard chemometric methods including Principal Components Analysis
• a machine learning approach, genetic programming
• a new multiobjective clustering method
• integration of results with high confidence protein-protein and genetic interactions
• the use of contraints-based modelling software to compare experimental and in silico results (Förster et al. 2003)

The challenges encountered in this work, e.g. the collection and instrument analysis of biological data and the selection of appropriate data pre-processing and analytical methods, are typical of those encountered in many ‘omic’ studies. From this data hypotheses can be tested experimentally, results integrated with those obtained from proteomic and transcriptomic studies and incorporated into existing models to gain more biological understanding of gene function. These are first steps in trying to build towards achieving the core aim of system biology, the wish to understand the whole and build quantitative models that will allow the investigation of dynamic systems.
Acknowledgement The authors would like to thank the BBSRC for their financial support.
Allen, J., et al. (2003). Nature Biotechnol., 21, 692
Dunn, W., et al., (2004), TrAC, In Press
Förster, J., et al. (2003), Genome Res., 13, 244

P-P03 Genetic network model for the AP-1 system

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The AP-1 system is a dimer composed by different transcription factor families. Activation of signal transduction pathways as cellular and oxidative stress, DNA damage, antigen binding in lymphocytes, and cytoskeleton rearrangement converge to it. Therefore AP-1 system is centrally involved in different phenotypic responses such as differentiation, proliferation, cell arrest, and neoplastic development. Here, we model the combinatorial genetic network that is derived from the activation of this AP-1 transcription factor as an additional component of cellular information processing.
While AP-1 network architecture was reconstructed by scanning of the literature we establish it dynamics by correlating cellular stimulus with experimental transcription expression profiles of their related genes and their different cellular phenotypic responses. To reproduce the activation over time of the mentioned transcriptions factors we model them with nodes of Continuous Time Recurrent Neural Networks (CTRNN). CTRNN have been shown to approximate the trajectories of any smooth dynamical system. For evolving the CTRNN, a simple variation of a Genetic Algorithm is utilized in order to achieve with the multi-objective optimisation problem.
Our modelling approach successfully can reproduce the dynamics of the AP-1 system. Together with additional experimental data, the genetic network model of AP-1 will be used to clarify and predict the central role of AP-1 as a key player in the signalling system of the cell.

P-P04 Pathways to analysis of microarray data

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Microarrays are increasingly used to profile genome-wide gene expression. A common aim in the analysis of gene expression data is the integration of array data with biological annotations, in order to identify pathways (such as metabolic or signalling pathways), or functions (such as those from the gene ontology) that are co-ordinately regulated. Several methods of pathway analysis quantify the overrepresentation of pathway annotations in genes displaying a particular expression pattern. These include calculating a 'z score', based on the observed number of matches between a gene list and a pathway and the expected matches, and 'gene set enrichment analysis' (GSEA), which involves comparing a pathway to a ranked list of microarray data. We compare these two methods of analysis on the same microarray dataset in order to investigate their differences. Broadly, the same pathways are indicated as overrepresented according to the two contrasting methods, but GSEA tends to find more significantly regulated pathways.

P-P05 Multiscale modelling of a cell

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The relevant information of the functioning of a cell covers several orders of magnitudes in time and length scales. For such a reason, simpler models based on ordinary (stochastic) differential equations (ODEs, SDEs) are used to simulate the emergent macroscopic behaviour, rather than more fundamental molecular descriptions. This approach is based on a vertical integration of models where the coefficients of the ODEs/SDEs are representative of the molecular information [1]. At a more detailed level, molecular dynamics (MD) and Brownian dynamics (BD) simulations can be used to compute these coefficients which are fed into the differential equations. In this case, the computational requirements are much higher and the complexity of the codes is such that appropriate coupling frameworks have to be used. Systems Biology Workbench (SBW) furnish a way to expose these models as 'remote procedures', which can be interfaced and coupled to describe higher level cell functions. However, due to the large computational cost, a possible interesting approach is to prepare such simulations in a way that they are exposed as GRID services [2] which can be accessed by ODE cell models to explore functional effects of microscopic mutations. With RealityGrid middleware for GRID computing, the computational resources are not assigned in advance and the effort required to expose the simulation as a GRID service is minimum. In particular, we look at a coupled molecular dynamics model and fast methods for the calculation of the chemical potential [3] which open a larger window on equilibrium and non-equilibrium MD simulations and has potentially interesting implications for the simulations just mentioned. In perspective, it would interesting to integrate these GRID services (such as BD and MD simulations, ODE solvers, etc) in order to explore the macroscopic functional effects at several levels of descriptions of a cell.

[1] Integrative Biology project, www.integrativebiology.ox.ac.uk;
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[3] R. Delgado-Buscalioni and P.V.Coveney, Phys. Rev. E 67, 046704 (2003);
G. De Fabritiis, R. Delgado-Buscalioni and P.V.Coveney, publishing J. Chem. Phys. (2004) (www.integrativebiology.ox.ac.uk/publications/JChemPhyspaper.pdf)

P-P06 A genetical genomics approach to gene network inference

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One of the challenges in systems biology is to discover the interaction structure of biochemical systems. We here propose to use a systematic approach to infer gene networks using several types of experimental data and consisting of several steps. The experimental data used in this approach consist of gene expression profiles and a detailed genomic marker map of multiple individuals in a segregating population; genetics and genomics combined. The steps are:
(1) Genome-wide QTL analysis of gene expression profiles to identify eQTL ('expression' quantitative trait loci) confidence regions
(2) Identification of regulatory candidate genes in each eQTL region
(3) Directional links are established from regulatory candidate genes to genes affected by the eQTL
(4) Statistical validation and refinement of the inferred network structures using Structural Equation Modeling.
The rationale behind this approach is that each gene's expression level can be treated as a quantitative phenotypic trait and used in conventional QTL analysis to identify chromosomal locations (QTLs) affecting it. In step 1 we thus find chromosomal locations (eQTLs) having causal influences on the expression of particular genes. In step 2 we identify genes located in the eQTL regions. The genes located in these eQTL regions are potential regulators of the genes whose expression is affected by these eQTLs. We can then propose causal links between regulator (located in the eQTLs) genes and the regulated genes (affected by the eQTLs)(step 3). The QTL analysis may suggest an encompassing network from all identified regulatory interactions, as well as candidate links which may be indirect and, hence, eliminated. In the final step, alternative models are tested using Structural Equation Modeling, a statistical technique for evaluating linear interaction models. In this technique, models are scored based on how well their model-implied covariance matrix matches the observed covariance matrix. In my presentation I will illustrate and evaluate this strategy using a series of simulated data.

P-P07 A dynamic model of cAMP signal transduction in yeast

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Yeast cells possess a number of signaling pathways to integrate information about nutrient supply with cellular growth and proliferation. The signaling route mediated by cyclic AMP (cAMP) and protein kinase A (PKA) regulates a large number of targets both at the posttranslational and transcriptional level in response to changes in carbon source availability. By affecting both metabolic processes and the cell cycle machinery, it also serves to coordinate cell growth and division. Measurements were performed in synchronous cultures and in oscillating continuous cultures of Saccharomyces cerevisiae to analyze the cell-cycle dynamics of cAMP and energy metabolism. A modular single-cell model integrating cAMP signaling with descriptions of the cell cycle machinery and central carbon metabolism is currently under development. This single-cell model will permit to simulate cellular behavior resulting from the joint action of metabolic and signaling networks during the yeast cell cycle. The present contribution focuses on two models of cAMP signal transduction, which can be used as exchangeable submodules in the integrated model. On the basis of an extensive literature survey, two dynamic models of the cAMP signalling pathway were developed, both of which provide a comprehensive description of the current knowledge, but differ in the level of detail. They account for stimulation of adenylate cyclase via Ras and a GPCR system, cAMP destruction by phosphodiesterases, (in)activation of PKA, and for the negative feedback exerted by PKA on its own activity. Results of the above-mentioned experiments were employed in combination with literature data and stability constraints to estimate model parameters. As a starting point, protein levels determined in a genome-wide analysis [1] served as estimates of the initial values of model species. Simulation results of both the small-scale model (20 reactions) and the large-scale model (400 reactions) will be presented and compared to experimental findings. The models provide a basis to address open questions regarding the underlying network structure and dynamic behavior of this signaling pathway. Plus, they can serve as a tool to identify suitable experimental conditions to efficiently discriminate between alternative hypotheses. Future work aims at incorporating spatial information and transcriptional regulation of key components of the cAMP pathway into the model. [1] Ghaemmaghami, S., et al. (2003) Nature 425(6959): 737-741.

P-P08 Metabolic quorum sensing: experiments with *S. cerevisiae*

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Populations of yeast cells in a homogeneous environment are able to synchronise their metabolic activity. Such a phenomenon can be viewed as a possible step in the development of multicellularity. The mechanism by which such collective periodic oscillations arise is however not yet fully understood.
In order to explore the nature of cell coupling, we performed experiments on yeast cells suspensions at different cell density. Using Stuart-Landau formalism, the microscopic state of the population is deduced by studying the response of the metabolic activity to perturbations and forcing with acetaldehyde.
A transition to incoherent oscillations was expected for low densities. The analysis of our experiments instead point to a different scenario, where each individual cell stops to oscillate when the cell density falls below a critical value. Thus, our experiments suggest that metabolic oscillations can sustain a quorum sensing-like mechanism in eukariotes.

P-P09 Phylogenetic analysis based on structural information of metabolic networks

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In this work we propose a method to extract phylogenetic information based on structural properties of the metabolic systems of different organisms. Such information on the relatedness of different species is commonly depicted in the form of phylogenetic trees.
In the literature there are many examples of the reconstruction of phylogenetic trees. Regularly, the data source on which such calculations are based upon are similarity measures between genomic, tRNA or protein sequences. In this way, hypotheses on the evolution of specific enzymes or genes can be developed. Rather than focussing on single enzymes or genes, in this work we propose to use as a data source information regarding the complete metabolism of an organism. Based on metabolic pathway information of over 160 species contained in the KEGG database, we identify the number and types of organisms in which each reactions occurs. This information allows to draw conclusions on which pathways are widely conserved, and therefore can be assumed to have appeared early during the evolutionary history of metabolic pathways, and which pathways are specific to certain organisms and can therefore be considered to have appeared later.
Also based on the pathway information from the KEGG database, we define various distance measures, use these to calculate phylogenetic trees, and compare their structure. Already simple distance measures such as the number of reactions which are different in two organisms result in trees in which related organsisms are generally grouped together. A more elaborate definition of the distance measure is based on the principles of network expansion presented by Handorf et al. This method describes the emergence of complex metabolic networks from simple structures. We claim that the proposed measure based on expansion processes more realistically reflects the evolutionary distance between metabolic networks since it is based on elementary mutation steps altering the network structures.

P-P10 Modelling of Drosophila segmentation gene expression with and without usage of attractors

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We have found recently that explicit representation of nuclear structure of Drosophila embryo is not necessary for modelling pattern formation in Drosophila segment determination (Gursky, V. V., et al. (2004) Physica D, v.197, p.286). This conclusion follows from the fact that correct pattern dynamics can be obtained in both spatially discrete and spatially continuous models. In particular, the successive nuclear divisions occurring in the embryo during this developmental time appears to be not connected to pattern formation in segmentation gene expression, because different schemes for mitosis lead to qualitatively the same results. One of important features of the considered models (both discrete and continuous) is that solution stays far from steady state (attractor) at all times. We study the discrete model modified by an assumption that final expression patterns are close to an actual attractor of the dynamical system. Parameter values (elements of a matrix of interactions between genes and rates for synthesis, decay, and diffusion of gene products) in the equations are found by fitting numerical solution to expression data. Advantages and drawbacks of using attractors to model gene expression patterns are shown in this specific system. We also demonstrate that in terms of attractors mitosis can play a dynamical role, providing a natural selector in multiple steady states which are potentially possible for the gene circuit.
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P-P11 Discovering compound mode of action with CutTree

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Defining the primary targets of chemical compounds and thereby improving the selection of which targets to be further evaluated in animal models and clinical trials (Phase I- III) has for long time been a key issue for pharmaceutical companies because improving the accuracy of this selection process will be extremely cost-effective. To reveal the mechanisms of action of a drug, it is necessary to identify the primary affected genes, the PAGs. We have developed an algorithm, CutTree, which distinguishes the PAGs from the secondary downstream effects of a chemical compound. CutTree provides an integral experimental design of cellular perturbation experiments and whole-genome expression measurements. Unlike previous methods (Gardner et al., Lum et al.) CutTree does not depend on the availability of whole-genome deletion libraries or a complete map of the gene network architecture. The efficacy of CutTree, compared to strategies depending on whole-genome deletion libraries, increases with the number of primary affected genes. An experimental validation reveals that CutTree discovers 80 % (4/5) of the primary targets of galactose in yeast using 17 samples of micro-array data. This success rate is almost identical to our predicted CutTree accuracy of 70 % in the case of five primary targets.
1 Gardner, T, et al., (2003), Science 301, 102.
2. Lum, P, et al., (2004), Cell 116, 121.

P-P12 Scopes: A new concept for the structural analysis of metabolic networks

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In this work we present a new method for the mathematical analysis of large metabolic networks. Based on the fact that the occurrence of a metabolic reactions generally depends on the existence of other reactions a series of metabolic networks is constructed. The algorithm iteratively calculates subsequent network generations by adding in each step those reactions whose substrates can be provided by the network of the previous generation. In our case, the reactions which are eligible to be added during the process are taken from the KEGG database. The course and the final result of this network expansion is strongly dependent on the initial substrates. We define the set of compounds which can be reached by a network expansion starting from some initial substrates as the scope of these substrates.
The concept of scopes can be used to answer the question which compounds can in principle be synthesized from a given set of substrates using a specified set of biochemical reactions. Moreover, we can identify reactions which are essential for these syntheses by analyzing the robustness of the scopes against deletion of reactions. The method can also be used to identify small chemical building blocks from which a majority of all compounds can be synthesized. We could show, for example, that almost half of the cellular compounds can be synthesized from the building blocks carbon dioxide, ammonia, sulfate and phosphate together with water. An analysis of the expansion process revealed crucial metabolites which can influence the expansion process dramatically. In this way we could show that common cofactors such as NAD^+, ATP, and Coenzyme-A facilitate the incorporation of a large number of reactions in subsequent generations.
The scope also represents a functional aspect of metabolic network itself. In this way, we can answer the question to which extend different organisms can use the available chemical resources. This information can be used to determine evolutionary advantages of certain organisms in different chemical environments.
We expect that the expansion process shows features characteristic for the evolution of metabolic systems. We may argue that the dependence of reactions on substrate availability implies a certain temporal order in which the different reactions may have appeared during evolution. This gives reason to assume that the history of the evolution of metabolism can be deciphered from current metabolic networks.

P-P13 Inferring gene regulatory relationships from time series microarray data based on the trend of expression changes

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The availability of time series expression data opens up new possibilities to study the regulatory and dynamic relationships among genes via reverse engineering. The current approach used in literature for reverse engineering of microarray data mainly focuses on inferring the regulatory relationships between genes from point-to-point expression values of gene pairs. Many of the important features of gene expression such as noises and time-shift of expressions cannot be properly considered by this conventional approach. To consider time-shifted and inverted gene expression profiles, Qian et al (J.Mol.Biol.,314,1053, 2001) proposed a local clustering method. However, this method still suffers from problems such as large local noises and calculation of time-shift point-to-point expression values. Here, we present a novel method to infer the regulatory relationships which is based on extracting the main characteristics of trends of expression changes between genes and is therefore more noise-tolerant. The method includes two major steps: first calculate the trend score and trend correlation coefficient among all the significantly expressed genes and then to infer their relationships based on a comprehensive index of these parameters. We applied this method to cell-cycle expression time series data of yeast. We detected many additional correlated gene pairs beyond those resulting from conventional correlation algorithms including local clustering analysis. We then examined many of the inferred gene pairs which have significantly correlated change-trends. Some of them are well-documented in literature which supported our predictions. For instance, we found a significantly correlated change-trend between the gene REB1(mainly as RNA polymerase II transcription factor or Pol I transcription termination factor)and MF(ALPHA)2.This is confirmed (with a p-value of 7.4e-4) by the genome-wide location analysis of Lee et al (Science, 298,799, 2002). We also found many new gene pairs with significantly correlated change-trends the functions of which are unknown. Based on this we could suggest possible regulatory relationships for experimental studies. Thus, this method is useful for inferring regulatory relationships from time series microarray data and for extending the co-expression network inferred by conventional clustering and local clustering algorithms.

P-P14 Secondary metabolites can create coexistence in the chemostat

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For microbial species competing for one limiting resource in a chemostat, mathematical models and in particular the competitive exclusion principle (CEP) predicts survival of only one species in any case. Quantitative experimental data from our model system related to the genetic disease Cystic Fibrosis alludes to the coexistence of at least two competing species. We developed a new mathematical model (extension of the classical chemostat) to comply with the experimental phenomena by including species specific properties of the microorganisms of concern.
We will present the mathematical tools and the analysis of the mathematical model, consisting of a four-dimensional system of nonlinear ordinary differential equations, as well as computed simulations for experimental data.
We found that the dynamic of the system changes in a fundamental way, if interspecific competition is included; a Hopf bifurcation occurs for an appropriate choice of parameters.
Experimental data serve as basis for the assumptions. These are a) one species produces a secondary metabolite, b) the metabolite has a growth-inhibiting effect, but can also be exploited as a secondary carbon source, c) some of the species could compete directly (e.g. via toxin production), and d) a lethal inhibitor could be introduced that cannot be eliminated by one of the species and is selective for the stronger competitor.

P-P15 Two Numerical Model Analyses for the Movement of a Restriction Enzyme.

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The inner cellular environment restricts the dimension of molecular diffusion. The environment is different from the idealized free reaction space condition. Defining fundamental reaction formulas are essential for faithful modeling and simulation of intra-cellular biochemical processes. In this study, we chose the EcoRV diffusion process to investigate the dimension restricted reaction.
There are 3 kinds of hypothesis for the targeting mechanism of EcoRV: sliding, hopping, and jumping. The first 2 is highly correlated process that means the dimension of the molecular diffusion is restricted, and the last is uncorrelated process.
In this study, we analyzed the EcoRV diffusion process by 2 different numerical models to estimate which manner is realistic as the targeting mechanism of EcoRV.
First, we tried to test by theoretical method for restricted reaction if the targeting process is correlated or not. This analysis suggested highly correlated process might be included in the targeting process.
Next, we tried to express the more detailed behavior of EcoRV with the stochastic model, containing an approaching, an association, and a sliding step by random walk.
This model could find 2 kinds of answer for reconstructing the experimental results [1].
One is the case the enzymes slide along on DNA or dissociate far away from the substrate. The other is the case the enzymes are well restricted around the substrate DNA but not always slide along the substrate DNA, and repeat hopping.
To explain the experimental results of with catenane [2], the latter case is supported because dissociation/re-association by hopping is required for the facilitation of the digestion of the target site in catenane.
In our stochastic model, the enzymes can slide less than 40 bp by random walk. This result agrees with the work based on a diffusion model [3].
These numerical analyses suggest that the diffusion manner of the EcoRV does not consist of long 'sliding'. At the same time, the diffusion manner is highly correlated and does not allow frequent 'jumping'. The most major diffusion process is suggested as 'hopping'. This study will be applied for the analysis of the dimension restricted biochemical reactions, which have occurred inner-cellular environment.
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P-P16 A reductive approach to analyze stochasticity in intracellular networks

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The stochasticity in behaviors of intracellular chemical reaction networks has been attracting much attentions from both theoretical and experimental viewpoints. However, roles of stochasticity in intracellular networks are not understood enough. From the theoretical viewpoint, one of the important issues for understanding stochasticity is how to evaluate fluctuation in molecular species involved in intracellular networks. This issue is not yet solved mainly because chemical reactions can work as the sources of fluctuation and the propagators of fluctuation simultaneously and they are inseparable. In addition the feedback structures in the intracellular networks makes it more difficult to understand how the fluctuation of molecular species is determined by the combination of the two roles of chemical reactions.
In this work, we develop a graph-based method named ``stochastic network analysis'' that were proposed by the authors to evaluate the fluctuation of intracellular networks. The method is designed to facilitate us to elucidate how fluctuation of a molecular species involved in an intracellular network is determined by the reactions in the network and the structure of the network. The stochastic network analysis is essentially composed of cumulant evolution equation and stochastic network graph that is a graph representation of the cumulant evolution equation. The stochastic network graph facilitates us to capture how fluctuation is generated and propagates into an intracellular network. The formulation of the cumulant evolution equation enables us to mathematically characterize the generation and propagation of fluctuation, which is intuitively understood with the aid of the stochastic network graph. In addition, by applying the decomposition of sources of fluctuation and transformation of stochastic network graphs, we can separate the roles of chemical reactions to generate fluctuation and to propagate fluctuation. Furthermore, the theory of signal flow graph and the contraction of stochastic network graphs provide us with a mathematical representation of fluctuation of a molecule in such a way that it is tightly associated to the structure of a SNG. By using the stochastic network analysis, we analyze fundamental mechanisms to attenuate fluctuation of molecular species, and demonstrate how the stochastic network analysis facilitates our understanding of the stochasticity in intracellular networks.

P-P17 Linlog Modeling Approach: Theoretical Platform for System Biology

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The advances in experimental protocols for sampling and measuring various cell components, i.e. intracellular and extracellular metabolites, amino acids and proteins, genes and transcripts lead to the generation of enormous amounts of data on biological systems. This raises the question of how to utilize these data to extract information on the functioning of the biological system. Modeling of the biological system offers a good tool to answer question. By developing a good model of a biological system and validate it using available data, we can enhance our understanding of what actually happens in the system under condition where the data is collected. Once the model is validated, we can use it to predict what is going to happen in our biological system under a particular condition by performing in-silico simulations. The challenge is how to develop a good model from available data.
In the last 10 years our group has been working on the development of an experimental platform for metabolomics: short-term perturbation experiments, sampling techniques, sample processing and LC-MS analysis of metabolite levels. On the theoretical side, we have introduced the linlog kinetic model, a simple-standardized-approximative way to model the kinetics of a biological system. The linlog kinetic model has been shown to have a good approximation quality using relatively few model parameters (1,2). My presentation will focus on the method development for kinetic parameter estimation from transient metabolite data using the linlog kinetic model (3). The method developed has been tested in a case study and has been shown to be simple, robust towards error in the data originating from sampling or measurement inaccuracy, and can accommodate various type of perturbations, either in metabolite levels of in enzyme activities.
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P-P18 Knowledge discovery by integrated analysis of metabolic and regulatory networks

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Recent studies on genome scale biological networks have revealed several inspiring structural and functional properties of these complex networks at systems level. However, relatively little has been done on an integrated analysis of these different networks. We recently reconstructed the transcriptional regulatory network (TRN) of *E. coli* which contains 1278 genes. Among them, about 400 genes code for nearly 300 metabolic enzymes. We then mapped these regulated enzymes on the reaction graph of the genome-based metabolic network of *E. coli*. This allowed us to identify about 100 missing links (unregulated reactions, most of their neighbors are regulated). These missing links turned out to be promising candidates for discovering new regulatory interactions. Using the arginine metabolism system as an example, two genes (*argA* and *argG*) in the arginine synthesis pathway are predicted to be regulated by the transcription factor ArgR because all the other genes in this pathway are repressed by it. This prediction is further strengthened by the finding that both *argA* and *argG* have the binding site for ArgR. This result promotes us to develop a new strategy for reverse engineering of TRN from metabolic phenotype, namely the reconstruction of a reasonable TRN to make metabolic pathways work properly under different conditions. Through this strategy, more regulatory interactions can be discovered for other functional systems.
On the other hand, integrating regulatory information into the metabolic network can improve the annotation of metabolic enzymes. For the arginine metabolism system, three enzymes in the arginine uptake pathway are found to be wrongly annotated in KEGG but rightly in Ecocyc. Furthermore, we found that among the three pathways from glutamate to ornithine, only the genes in the longest pathway are co-regulated with the genes in the pathway for converting ornithine to arginine. This implies that in reality the longest pathway rather than the shortest pathway are used for arginine synthesis. We further show that the longest pathway is also the most energy consuming pathway. This challenges a basic concept of flux balance analysis which predicts metabolic fluxes often by assuming a maximal energy or biomass production as the optimization objective. In summary, an integrated analysis of metabolic and regulatory networks is important for understanding both cellular metabolism and its regulation and essential for systems biology.

P-P19 Modelling and simulation of dynamic signals in cells

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The transduction of external and internal signals from cell membrane and other cell organelles to the nucleus is a dynamic process, enabling the cell to react and to adapt to changes in their environment. Feedback loops inside the complex biochemical networks leads to a complex spectrum of response signals, e.g. reversible and irreversible switches, stable and instable oscillations.
With systems of nonlinear coupled ordinary differential equations we investigate dependence of the dynamic response on parameters, missing or additional feedback loops.
The role of fluctuations in biochemical signalling networks we study in the framework of Monte-Carlo simulations techniques and compares the results with corresponding ODE-modells.

P-P20 Systems analysis of yeast glucose sensing system

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In the budding yeast *S.cerevisiae*, expression of glucose transporter genes (*HXTs*) are regulated by glucose. Especially, the system that induces the expression of *HXTs* is called “glucose induction system”. In this system, glucose sensors (Rgt2 and Snf3) ultimately regulate the activity of a transcriptional repressor (Rgt1), through mediator proteins (Mth1 and Std1) and ubiquitin ligase (SCF^{Grr1}). We recently obtained evidence that Rgt2 glucose sensor activates casein kinase I (Yck1 and Yck2) on the plasma membrane, then the activated casein kinase I phosphorylates Mth1 and Std1. This phosphorylation triggers the ubiquitination and degradation of Mth1 and Std1 (1). In this condition, Rgt1 is phosphorylated and lost its DNA binding activity (2). In addition to these, genome-wide microarray analysis revealed that the whole regulatory network structure of this system including the auto-regulation (3). From these knowledge, we made a mathematical model using biochemical process diagram editor software CellDesigner (4) and simulator softwares, and studied about the possible characteristics of the glucose sensing system.

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P-P21 Investigating the structure of integrated biological networks

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Theory of complex networks provides an intuitive setting for studying biological relationships at the cellular level and beyond in the topological context [1]. It has already been suggested that the topological properties of networks relate to biological function [2]. However, due to diversity of biological relationship types even at the molecular level, studying the biological network topology at one level only (e.g. metabolic networks) may miss much of important information about cross-talk across multiple pathways and potential feedback loops via regulatory networks. Our aim is to develop a framework to study topology of multiple biological networks.

As a start, we mapped KEGG, TransFac, TransPath, MINT, and BIND databases using XML [3], with example shown in Figure1. We developed a Java-based tool that allows parallel retrieval across multiple databases, incl. metabolic pathways, protein-protein interactions, signalling and regulatory networks. The results are then visually displayed as a network (Figure2). Edge attributes contain information about type of relationship, possibly quantitative or semantic information (such as *is located in* in case of linking a protein with a complex entity such as cell organelle).

Information in integrated network form is a starting point for deeper topological and functional mining. Presently, we are primarily interested in the concept of similarity, i.e. how to define a distance metric across multiple networks. We applied various distance metrics and nonlinear mappings into lower-dimensional space such as Sammon’s mapping and self-organizing maps, of which results will be presented.

We are also extending our approach to more complex entities by combining existing pathways with the automated text mining. We believe our approach will provide a powerful framework for context-based mining and modelling of biological systems.

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P-P22 An *in silico* model for the optimization of threonine production in *Escherichia coli*

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Threonine is an essential amino acid for birds and mammals and so there is considerable interest in its economic industrial production for a variety of uses. Its industrial production is insured by *Escherichia coli* overproducer strain. We have adapted and improved pre-existing dynamic model of central metabolism and threonine pathway by Chassagnole *et al*. (Biochem. J., 2001, 356,415-423 and Biotechnol. Bioeng., 2002, 79(1):53-73) to create a global *in silico* model of such a production strain.
The aim of this work was to obtain strain improvements in the threonine production by DNA recombinant techniques directed to the controlling enzymes in the threonine synthesis. A study of the modification effect of the key branch points of the network (PEP and G6P node) provides us enough information to predict changes on threonine production. The model has been implemented with experimental results (enzyme activities, flux measurements and intracellular metabolite concentrations) from a bioreactor cultivation.
The first part of the modeling procedure was the estimation of kinetic parameters (Michaelis and inhibition constants and rates maximum for each enzyme) by comparison between simulations and experiments. Then, we simulated the "*in vivo*" behavior of threonine production for identification of metabolic targets for genetic modifications. Finally, we checked the efficiency in threonine production of the optimized strains.

P-P23 Kinetic models of phosphorylation cycles: the role of protein-protein interactions

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Activation-inactivation cycles catalysed by kinases and phosphatases are a core component of cellular signal transduction. Usually phosphorylation reactions have been mathematically treated assuming Michaelis-Menten kinetics and mostly disregarding the role of phosphatases. In signaling networks, however, the substrates of many reactions are kinases and phosphatases themselves, and the balance between activating and deactivating enzymes constitutes a key aspect to be considered. Therefore, we have developed a systematic and general analysis, which, in particular, resolves the kinetics of kinases and phosphatases equally. The model accounts for the formation of enzyme-substrate complexes and the subsequent modifications of a single or several phosphorylation sites in the target protein.
Two main parameters shape the stimulus-response curves in the model: the degree of saturation of kinase and phosphatase with the target protein and the extent of inhibition exerted by binding of the respective reaction products to the enzymes. By changing these parameters, the response curves were compared with respect to the stimulus sensitivity measured through the response coefficient. As result, well-structured "phase diagrams" of the phosphorylation cycles were obtained. There are two regions of high sensitivity corresponding to the parameter ranges of zero-order ultrasensitivity, on the one hand, and strong product inhibition with low enzyme saturation, on the other. Moreover, higher stimulus sensitivities are particularly observed in the phase diagram of proteins modified at several residues. In these proteins, a bistable response can arise in the region of zero-order ultrasensitivity when the phosphorylation sites are cooperatively modified.
We also computed in such phase diagrams the response time to reach a steady-state phosphorylation level of the substrate after a stimulus change. The result demonstrates that this transition time also strongly depends on the kinetic design of the phosphorylation cycle. The most influential parameter is the substrate saturation of the enzymes: saturation cycles are generally slower than unsaturated ones. Finally we addressed the effect of the order of phosphate processing on multiply phosphorylated proteins. Response curves in a random phosphorylation are shallower but with a faster kinetics compared to the sequential case.
Salazar C. and Höfer T. (2003), J. Mol. Biol. 327, 31. Salazar C. and Höfer T. (2004), FEBS Lett., in press

P-P24 Modelling transient dynamics of osmo-stress response in Yeast

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A special focus of System Biology has been the modelling of cellular signalling processes. Especially the MAP kinase signalling cascade that is widely conserved among eukaryotic cells has been studied intensively in theory and practice. Theoretical studies of MAP kinase cascades often concentrate on their steady state properties, like, e.g. ultrasensitivity or oscillations. It has been noted that for MAP kinases to exhibit ultrasensitivity a distributive double phosphorylation is one possible mechanism. For few systems it was proved that this is actually the case. In many systems, e.g. in yeast, MAP kinase cascades are neither the only components of a signalling cascade nor are they isolated from other cellular compounds. It is rather the case the MAP cascades are usually preceded by other signalling constructs, *e.g.* G-protein complexes or phospho-relay systems and are often recruited to scaffold complexes where a distributive phosphorylation has not been demonstrated, yet. Thus, on the one hand it is possible that ultrasensitivity is achieved by other parts of the signalling cascade than the MAP cascade and one the other hand steady state properties might not be so important when it is the transient dynamics that are decisive as, *e.g.*, in stress response. From the practical point of view it is desirable to have models with as few reactions as possible because data to fit parameters is often scarce and models are usually underdetermined. Addressing these issues we will present a modelling study of the Sln1 branch of the HOG pathway in Yeast that consists of a three-compound phospho-relay system and a three-step MAP kinase cascade. We fit several possible model structures of the Sln1 branch to measured data of transient Hog1 activation upon several osmotic shocks. We explore the influence of possible model structures on the resulting fit with special emphasis on a) the effect of model structure, e.g. processive versus distributive phosphorylation, on transient dynamics, b) the effect of scaffolding and c) steady state properties. Moreover, we examine the performance of several global optimisation algorithms to fit the measurements to the model parameters.

P-P25 Inferring regulatory networks from experimental data

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Standard methods for the analysis of microarray data are often emphasizing on the identification of single genes within the process of interest only and thus neglecting important information like the time dependencies hidden in the data sets. From a systems biology point of view it is therefore necessary to develop a new class of analytical methods. One major aspect that has to be addressed by these new methods is to understand the regulatory mechanisms within a cell, *i.e*. the structure of regulatory networks and the corresponding kinetic parameters. Those methods must be able to cope with the high complexity of the regulating system and the ambiguity in the data. Additionally, they have to comply with biological constraints like the stoichiometry of biochemical reactions or known interactions of system components. Over the last year, we developed a software framework that aims to infer gene networks from microarray data and also metabolic systems from experimental data. The framework comprises known models for simulating regulatory networks like linear weight matrices, non-linear S-systems and arbitrary differential equations as well as models that were developed in our research group like pseudo-linear weight matrices. For the estimation of the model parameters, we developed new algorithms primarily based on evolutionary algorithms (EAs) including evolutionary strategies, genetic algorithms and genetic programming or on direct heuristics. Our developments include massively parallel EA implementations, hybrid optimization approaches combining EAs with local parameter searches and also multi-objective exploration of alternative network structures. Additionally, these algorithms automatically incorporate biological knowledge from public databases like KEGG to meet certain constraints to include a priori information of network topologies. Further on, we are experimenting with extended solution representations to increase the performance of the simultaneous optimization of parameters and topologies. The framework was successfully used on the inference problem of gene and metabolic networks and the project's website (*http://www.jcell.org*) gives a comprehensive list of recent publications.

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Spieth, C., et al., (2004), LNCS, 3102, 461 Spieth, C., et al., (2004), CEC 2004, 152
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P-P26 First steps towards a multi-dimensional iron regulatory network

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Iron homeostasis is central to many biological processes and thus imbalances in mammalian iron metabolism are associated with frequent iron overload and iron deficiency disorders. The iron regulatory network is a complex multi-dimensional network combining organ to organ communication (mediated by iron hormones), cellular interactions and biochemical pathways. This network can be addressed by several endogenous (*e.g.* cell cycle) and exogenous (*e.g.* infection) stimuli.
In order to define and ultimately visualize a complete iron regulatory network we produce and collect specific signature profiles derived from cell based and whole animal experiments and correlate them with biochemical, phenotype and physiological information. Gene expression profiles recorded on *IronChips* form the basis for our *in silico* iron regulatory network. *IronChips* are cDNA based microarrays that sensitively and accurately measure expression changes of genes involved in iron uptake, storage and recycling, as well as from genes involved in a number of interlinked pathways (e.g. NO metabolism, redox pathways, stress responses as well as acute phase and immunity). Systematic *IronChip* analysis of selected tissues derived from knock-out mice with defects in central players of iron metabolism inform us about the relationship of these genes within the network.
IronChip data evaluation and construction of such a multi-dimensional iron regulatory network requires the development of sophisticated algorithms. The recently developed IronChip Analysis Tools (ICAT) offers a solution for the automated data analysis associated with this specialized array platform.

P-P27 Nutrient starvation in baker's yeast, and the implication of protein degradation for Vertical Genomics

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In the Vertical Genomics program gene expression, protein synthesis, protein degradation and posttranslational modification will be related to metabolic fluxes and metabolite concentrations. This will be done by quantitative dynamic measurements, regulation analysis and kinetic modeling. Glycolysis in *Saccharomyces cerevisiae* is a good model system to test these relations, because it is one of the few pathways for which kinetic properties of the enzymes are known sufficiently to calculate the flux from the enzyme activities. Furthermore yeast can be brought under well-defined steady-state and transient conditions for a detailed quantitative analysis.
In this project different perturbations will be studied and nutrient starvation is one of them. During industrial production of beer and baker's yeast the cells often undergo periods of nutrient starvation. Starvation leads to a loss in fermentative capacity, due to a degradation of glycolytic enzymes, modification of the palette of expressed transporters, and due to altered topogenesis in some of the transporters. Fermentative capacity is an important characteristic for the application of baker's yeast in the dough. During washing, packaging and storage, the baker's yeast is subjected to complete starvation, while in the dough the cell is subjected to a nutrient-rich environment and has to start fermentation. Knowledge about the regulations at the various levels of the cellular regulation hierarchy should guide engineering towards improved fermentation capacity after a period of starvation. Therefore the dynamics of glycolytic genes and proteins during starvation will be measured and correlated to the response of the glycolytic flux and metabolite concentrations. Within the Vertical Genomics program the starvation project will be a test case to quantify the importance of protein degradation in the regulation of fluxes.

Power Posters

P-PoP1 New parameter estimation method with possible application in systems biology

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Parameter estimation is a research topic of large interest : from science and engineering to biology and medicine. One of the major problems is that the error function has multiple minima. Using our previous results on synchronization of chaotic systems we obtained promissing results[1] : a global minimum for the error function. We want to apply these general ideas to the specific problems of systems biology[2,3] with the focussed goal of improving the current software[2,3].

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P-PoP2 Effects of noise in metabolic flux analysis

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The quantification of metabolic fluxes are paramount to identify the cause-effect relationship between genetic modifications and resulting changes in metabolic activities. Given that intracellular fluxes are nonmeasurable quantities, careful experimental methods and associated estimation methods are needed to determine them. In metabolic flux balance, intracellular fluxes are calculated from measured extracellular fluxes and stoichiometric equations, coupled with 13C labelling experiment that provides additional information about the intracellular fluxes. The measurements are typically noisy and here, the aim of the study is to analyse the effects of noise in the estimation accuracy and to develop methods that compensate for these directly.
The flux estimation methods investigated belong to three different types. In the seminal work on metabolic flux analysis, the flux estimation problem was posed as a classical least squares problem. This and other variants of least squares methods form the first of these types. Second class of methods are based on computational intelligence methods such as evolutionary algorithms. The choice of evolutionary algorithms is motivated by the need for nonlinear optimisation methods that are required in the presence of noise. Finally, the third class of algorithms are based on stochastic approximation type methods.
Results will be presented for a small metabolic network consisting of the Cycle Pentose Phosphate pathway demonstrating the advantages of methods that attempt to compensate for noise directly.

P-PoP3 A new dynamic complexity reduction method for biochemical reaction networks

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Modeling and simulation of the dynamical behavior in cellular systems are often hindered by size and complexity of the underlying biochemical reaction networks. With the aim to facilitate the identification of dynamical key features of such systems and to aid a functional separation into subsystems we present a new dynamical complexity reduction method based on the concept of a time scale decomposition (TSD). The TSD-based method relies on the fact that biochemical processes typically take place on time scales differing by up to several orders of magnitude. In contrast to most existing complexity reduction methods - either based on the evaluation of structural network properties only or on specific dynamical assumptions (e.g. steady state) - our approach is independent of any specification for the dynamical behavior. Processes being sufficiently fast compared to the actual time scale of interest - which definitely changes in the course of the dynamical simulation - are assumed to be relaxed. Thus, the effective number of ordinary differential equations which need to be solved in order to obtain the time evolution of the reaction network in the context of a deterministic, homogeneous modeling formalism is reduced in an automated way. The reduced system dynamics at a given state is confined to a so called Intrinsic Low-dimensional manifold (ILDM) of the full state space. An analysis of the reaction species participating in the active processes provides valuable insight into the nature of the interactions that are responsible for the system dynamics on a specific time scale and may allow the identification of functional couplings within the network. The capabilities of the new dynamic complexity reduction method are illustrated in a study of the complex dynamics of a Peroxidase-Oxidase (PO) reaction network model. As a result, we managed to reduce the dimension of the active state space substantially in the course of the simulation, even in the difficult case of complex oscillatory system dynamics. For the first time a potential decomposition of the PO network into subsystems is shown to depend sensitively on the specific dynamics of the system.
Reference:
J. Zobeley, D. Lebiedz, J. Kammerer, A. Ishmurzin, U. Kummer, A new time-dependent complexity reduction method for biochemical systems, submitted to Transactions on Computational Systems Biology (2004)

P-PoP4 Determination of in vivo non-steady-state fluxes and kinetic information using stable isotope labeling and metabolite pool size data: theory and application

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Incubation of plant material in labeled isotopes often reaches neither isotopic nor metabolic steady state within the constrained time span of experimental measurements. This can be problematic since at a non-steady state, flux balance cannot be established. For this reason here we develop a mathematical approach to calculate fluxes within systems that do not approximate steady state. The approach is based on mass balance of all forms of molecules and employs trapezoid Euler's numerical method to deal with differential equations. The general method for deriving non-steady-state fluxes is established, and construction of overdetermined systems for non-steady states is proposed. Determination of fluxes based on GC-MS analysis is analysed in detail. We show that non-steady-state fluxes can be derived based on time-dependent metabolite pool size and specific labeling data. Moreover, when a number of data points are available, the approach is able to predict the dependence of flux on pool size of substrate and as such to reveal which enzymes do not follow conventional Michaelis-Menten type kinetics. Therefore, regulatory enzymes can be identified for further study. In addition, kinetic parameters can be estimated on the basis of this approach for Michaelis-Menten type kinetics. As a first example of the application of this approach we apply it to the analysis of primary metabolism in tuber discs isolated from wild type potato plants. We demonstrate that this approach is able to identify irrational flux combinations that violate mass balance, and as such allows a structural analysis of the metabolic network under evaluation. Despite the challenges presented by these findings certain fluxes including the interchange between glucose-6-P and fructose-6-P could be readily calculated. We discuss the potential of the approach both in analysing fluxes of metabolic networks and more generally within kinetic modeling strategies.

P-PoP5 An adaptive system approach for the modelling of genetic regulatory networks. Glucose metabolism study in the yeast.

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We have used a dynamic neural network to model the yeast glucose metabolism response to glucose deprivation in the culture medium. Our aim was to produce a predictive rather than explicative model, in order to address the question: “which molecule of the network should we act upon to obtain a given biological response?” The network was built from literature analysis and KEGG data and includes 133 molecules (3 metabolites, 99 enzymes, 26 transcription factors, 5 signal transduction proteins, connected through 516 interactions). The model was trained by DNA microarray data describing the gene expression response to the fermentation to respiration switch (De Risi et al., Science (1997)278:680-6). The simulation provides a hierarchy of the molecules classified in terms of relative distance to the biological response to be obtained. The model has been applied to the prediction of a gene knock-out response and the detection of the invalidated gene was within acceptable error margins. We will present our model and results and more specifically discuss the redundancy of biological regulatory mechanisms as arguing towards the use of adaptive models, and the impact of the network heterogeneity (scale-free structure) on the learning procedure and inferred parameters.

Tools and Methods

Posters

T-S01 Oscillatory mechanisms derived from phase and amplitude information

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Due to time-scale separation, a dynamical system close to a bifurcation will evolve according to the universal dynamics of that particular bifurcation. We have exploited this fact to devise a novel approach for determining the oscillatory mechanism for systems close to a supercritical Hopf bifurcation. In essence, the method works by identifying the chemical components of the two dynamical modes associated with the oscillatory dynamics: an activating mode and an inhibitory mode. There is no need for prior knowledge of the network structure, the only information required is measurements of the relative phases and amplitudes of the oscillating substances. Hence, metabolomics and mRNA arrays are ideal sources of data. The feasibility of the method is illustrated by its use for analysis of glycolytic oscillations in yeast cells.

T-S02 Application of modelling and simulation to drug discovery: The ErbB System

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The implication of the ErbB family in the pathogenesis of various cancers has made it a popular target for the development of targeted anti-cancer therapies. ErbB dimerisation, trafficking, and activation are complex processes, making it difficult to intuit how perturbations, such as drug intervention, will affect the system dynamics. We need computational approaches to keep track of and to quantify this complexity. AstraZeneca, in collaboration with the Lauffenburger lab at Massachusetts Institute of Technology, have developed a computational model implementing commonly accepted principles involved in ErbB signal transduction. The current ErbB model is made up of Ordinary Differential Equations (ODEs) and is based on detailed mechanisms of ErbB receptor interactions and downstream signalling components. It contains ~300 species, ~400 parameters and more than 500 reactions. A major challenge in dealing with models of this size is information management and model visualization. Text mining software is used to capture kinetic constants and models are displayed graphically using TeraNode™ Design Suite. Parameter estimation and sensitivity analysis are being exploited to assist model validation. The model is being used to predict the dynamics of receptor phosphorylation in the context of different cell lines and ligand environments. Recent work in our group has demonstrated that a deficiency in internalisation is sufficient to explain the observed signalling phenotype of the Gefitinib-responsive mutants found in NSCLC. Gefitinib ('Iressa'; ZD1839, AstraZeneca, Wilmington, DE) is an ATP-competitive small molecule inhibitor of ErbB1, approved for use in the treatment of non-small cell lung cancer (NSCLC). About 80% of Gefitinib-responsive tumours in NSCLC carry mutations in ErbB1. This model prediction has been experimentally validated using a Gefitinib-responsive and non-responsive NSCLC cell line. The Gefitinib-responsive cell line is shown to be deficient in the internalisation of two ErbB1 ligands, EGF and TGFa. This work provides a mechanistic basis for the link between the role of ErbB1 in oncogenesis and Gefitinib response through decreased internalisation of ErbB1 and increased signalling to AKT.

T-S03 Combined optimization technique for biological data fitting

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Motivation.Development of the organisms from embryo to the adult is one of the central unsolved problems of biology. We are working on characterization problem of systems biology of development in context of the segment determination gene network of a *Drosophila* embryo. While gene expression is evaluated at a time resolution of a few minutes and a spatial resolution of one cell (see FlyEx database), the regulatory parameters cannot be determined experimentally, and are to be found as the solution of the inverse problem by minimizing the deviation of the model output from the data. We apply a chemical kinetic model describing the dynamics of the expression patterns of the segmentation genes during the blastoderm stage by means of the system of **highly non-linear reaction-diffusion equations** *(Jaeger, J, et al., (2004), Nature, 430, 368)*.A random search technique, being extremely computationally intensive, is sometimes the only choice for finding the set of parameters that provides the best fit of model to data. Therefore the main problem is to reduce the complexity of finding the parameters of mathematical models.

Results. We developed the **Combined Optimization Technique** (COT) to reduce the computational cost of solution of the inverse problem of modelling. COT combines advantages of random search and gradient descent. Starting from an arbitrary initial set of parameters, a rough approximation of a minimum is found by the random search, namely, **Simulated Annealing** (SA), while the final solution is given by **Optimal Steepest Descent Algorithm** (OSDA), developed earlier *(Kozlov, K, et al., (2003), Techn. Physics, 48, 6)*, and successfully applied as the local optimizer in*(Gursky, V, et al., (2004), Phys. D, 197, 286)*. The dependence of COT convergence of the initial approximation and quality criterion is investigated and the strategy of transition from SA to OSDA is studied here. COT demonstrated high accuracy in reconstruction of model parameters and the **30%** total performance benefit in a two-gene network. Further study is performed currently to increase the speed up by application of new automated tuning methods for the OSDA part of COT. ***Acknowledgments:*** The support of the study by the NIH Grants RR07801, TW01147, the CRDF GAP Awards RBO685, RBO1286 is gratefully acknowledged.

T-S04 Systematic identification and characterisation of synthetic lethal interactions in the metabolic network of yeast

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To what extent and why do the effects of mutations depend on the genetic background? Do deleterious mutations act synergistically? What is the mechanistic basis of genetic interactions and how does it depend on the environment? Answers to these questions are relevant not only to functional genomics, but also to problems such as the evolution of sexual reproduction and how deleterious mutations are eliminated from the population.
Owing to the huge number of potential gene combinations, progress in answering these questions is, however, limited by the lack of efficient genome-scale experimental mapping of genetic interactions. To overcome this difficulty, we propose a combination of *in silico* and *in vivo* studies to screen for synthetic lethal relationships in the yeast metabolic network.
First, we apply flux balance analysis (FBA) to the genome-scale metabolic model of *S. cerevisiae* (Forster et al. 2003) to search for candidate gene pairs showing synthetic lethal interactions. Next, we use laboratory experiments to validate the model's predictions. Our preliminary results suggest that i) FBA is able to predict synthetic lethal interactions, ii) many of the interactions are environment specific, iii) although the density of interactions do not differ significantly between nutrient poor and nutrient rich growth conditions, we observe twice as many genes participating in synthetic lethal interactions in nutrient poor environment and iv) only about 20% of synthetic lethal gene pairs can be explained by the presence of gene duplicates (isoenzymes), this fraction, however, is significantly higher than the 2% previously reported for non-metabolic genes (Tong et al. 2004). The implications of these findings for genetic robustness and phenotypic plasticity are also discussed. ***References:*** Forster, J, et al. (2003) Genome Res 13, 244; Tong, AH, et al. (2004) Science 294, 2364.

T-P01 Genome-scale analysis of *Streptomyces coelicolor* A3(2) metabolism

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Streptomyces coelicolor A3(2) is by far the best genetically studied *Streptomyces* strain and has become a model organism for *Streptomyces* species. Release of the *S. coelicolor* A3(2) genome sequence has further expanded the knowledge of this organism and enabled the application of genome-wide analysis techniques like DNA arrays for transcriptome analysis and proteomics studies. We believe that integration of the genome-wide data through the use of mathematical models will enhance the extraction of information about the molecular mechanisms governing different processes in *S. coelicolor* A3(2). In this context it has recently been shown particularly valuable to use metabolic genome-scale models.
The metabolic network of the *Streptomyces* model organism - *S. coelicolor* A3(2) was reconstructed at the genome-level. The reconstruction was based on annotated genes, physiological and biochemical information. The network includes 823 biochemical conversions and 151 transport reactions, accounting for a total of 974 reactions. 700 of the reactions in the network are unique whereas the remaining reactions are iso-reactions. The number of metabolites in the network is 500. Seven hundred sixty nine (769) open reading frames (ORFs) were included in the model, which corresponds to 14% of the ORFs with assigned function in the *S. coelicolor* A3(2) genome. Flux balance analysis was used for studies of the reconstructed metabolic network and for assessing its metabolic capabilities for growth and polyketides production. The reactions essentiality was studied for growth on 63 carbon sources and 2 nitrogen sources; hereby the core of the "real" essential genes was identified. Furthermore, we illustrated how reconstruction of a metabolic network at the genome level could be used to fill gaps in genome annotation.
The ongoing project concentrates on generation of large-scale data (gene expression, proteomics and metabolomics) in *S. coelicolor* A3(2) and its regulatory mutant and on the integrated analysis of these datasets in combination with metabolic model.

T-P02 Relational learning of biological networks

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The last few years have seen a lot of different approaches for the reconstruction of biological networks. Those approaches essentially differ about the kind of data used and about the models of biological networks considered. The data used can be either numerical like time series generated by micro array experiments, or symbolic, like annotation databases or ontologies extracted from scientific articles by text mining algorithms. The models considered usually have graph structures and involve different kind of objects, like genes, proteins, metabolites or reactions. In order to deal with heterogeneous data as for representing highly relational models, it appears appropriate to infer biological networks with relational learning techniques.
As a proof of concept, we used the Inductive Logic Programming [1] system Progol to learn the concept of gene regulation based on gene expression data. The approach is the following:
-We consider a known gene network with associated expression data and we represent both in first order logic
-We learn a first order logic definition of gene regulation with Progol
-We try to discover potential regulations with the definition of regulation outputted by Progol
In order to represent expression data in a compact way, we discretized it in terms of expression levels, of variation directions and of time. The time discretization uses the notion of time intervals. We empowered the system with predicates able to capture relations between intervals, inspired by a formalism introduced by Allen in [2], which constitutes a new approach to deal with time series in ILP.
The system has been successfully tested on artificial datasets generated by different kinds of dynamic systems. It has also been tested on real datasets, one related to the SOS DNA Repair network of E. Coli and one related to the cell cycle of the Yeast. Current work follows three complementary directions:
-We try to use other sources of information like the Gene Ontology database or metabolic datasets
-We are working on new models combining gene regulatory networks and metabolic pathways
-We are currently investigating methods combining first order logic and probabilities (see [3])
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T-P03 A new Information System to manage and analyse information on biochemical interactions

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We are developing a relational database system for representing biochemical objects and their functions.
A major challenge of molecular biology is to understand how biochemical objects interact. There is a huge amount of publications, published every month, which provide new data on those interactions. We are able to automatically extract these information but the crucial question is:
Based on the bulk of existing data, what is the information gain we can draw from textmining results?
In order to approach this problem, we are developing a system for representing and analysing biochemical interactions.
In a second step we will try to draw useful conclusions by applying reasoning methods, based on classification co-occurences.

T-P04 Reduced order modeling of global regulation - redox regulation in *Escherichia coli*

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The amount of measurement data necessary to identify and validate detailed mathematical models of global regulatory systems makes it difficult to build such models. Nevertheless, modeling global regulation is helpful to understand the role of the involved regulatory proteins.
Therefore, there is a need for a modeling methodology capturing the key aspects of global regulation, without requiring the knowledge of the kinetic properties of all involved compounds.
As a case study we consider the global redox regulation of *E. coli*. With decreasing oxygen availability *E. coli* adapts its metabolism in four distinct phases from pure respiration to pure mixed acid fermentation. When operating in one phase the redox state as reflected by the concentration of NADH is kept constant, even if oxygenation changes (Alexeeva S. et al., (2003) J Bacteriol, 185, p204).
This indicates that despite the multitude of the actual signals of involved transcription factors (e.g. Fnr: O2, ArcAB: quinones) the underlying redox state of the cell is the relevant overall input signal for a controller that is able to stabilize the redox state at different setpoints. This simplified control structure is the basis for a reduced order model. The model is divided in a model of the controlled system, i.e. metabolism, and the controller, i.e. regulation.
The metabolism is approximated by a simple phenomenological model where all compounds except NADH and ATP are assumed to be quasistationary. The inputs to the model of metabolism are the concentrations of key enzymes (control inputs) and the environmental conditions (disturbances).
The regulation model uses the NADH and ATP concentrations (controller inputs) in order to compute the activities of the transcription factors via sigmoid characteristic curves. The transcription factors influence gene expression (controller output).
The resulting ODE model of metabolism and regulation contains only a relatively small number of dynamical state variables (ATP, NADH & enzyme concentrations) and kinetic parameters.
Simulation studies show good qualitative agreement with experimental data. For further validation of the model mutant strains have to be considered. An extension of the model for the study of other global regulatory systems will clarify the generalizability of this approach.
The introduced approach concentrates on the modelling of principal dynamic variables. It may be appropriate for the modelling of large regulated metabolic networks.

T-P05 Technical variance, quality control and scaling: necessary steps towards meta-analyses on large expression databases

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Objectives: High-density oligonucleotide expression arrays (GeneChips) enable the measurement of tens of thousands of genes in parallel. For every measured transcript (represented by a probe set on the chip) a Signal value is produced, which is ideally linear proportional to the mRNA concentration in the original sample. As the target measurement of a chip experiment, Signal values of course reflect the influence of biological variance sources, which are actually to be enlightened by the experimental design. Unfortunately technical variance sources disturb the Signal values in several ways. Even chips of good quality show influences of technical variance sources and have to be scaled for further analyses.

Result: As a fundamental framework for own analyses an SPLUS library for the interaction with the GeneChip technology was implemented. The first result of our work was the description and quantification of the occurring technical variance and insights about the main source of technical variance. To do so, a set of quality criteria was evaluated on several real-world data sets. The second result was the assessment of scaling methods towards their capabilities for the compensation of technical variance effects.

Conclusions: The presented work is an important step to make meta-analyses on large expression databases reasonable. Meta-analyses on publicly available expression data analogous to meta-analyses on publicly available sequence data like BLAST give insights about the expression behavior on a global scale. In addition, these meta-analyses open the field of system biology in mammalian organisms.

T-P06 Genomic rearrangements : influence of the genetic context on chromosomal dynamics

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Chromosomal rearrangements such as duplications, insertions and deletions contribute to the genome plasticity and represent a key event in evolution. They can also be involved in oncogenesis in pluricellular organisms. In our laboratory, a genetic screening based on a particular allele of URA2 gene in *Saccharomyces cerevisiae* was created thus allowing a spontaneous and positive selection of rearrangements such as duplications, Ty1 insertions or deletions.
In order to test the influence a context might have on appearance of chromosomal rearrangements, we selected revertants in *Saccharomyces cerevisiae* S288c haploid or diploid strains. Using molecular and genetic approaches, we determined the rearrangement responsible for the reversion event in 13 revertants. We first observed that the frequency of diploid or haploid revertants obtained in S288c context was higher than the one obtained in FL100. Moreover, no insertion of Ty1 transposon was observed in S288c whereas in FL100 this event occurred in half of the revertants. This suggests that FL100 and S288c strains have a different behaviour regarding revertants selections. Therefore genetic context is important for obtaining different chromosomal rearrangements both concerning the frequency and particularly the type of selected rearrangement.
S288c will facilitate the study of the mechanisms involved in chromosomal rearrangements appearance. The knowledge of the sequence should allow easier characterization of the chromosomal sites involved in these rearrangements such as the duplication sites. The S288c context has also been used for a collection of systematic genes deletion mutants that will help us to construct more easily isogenic S288c mutants in order to study the genes whose product is involved in mechanisms responsible for the rearrangements.

T-P07 CellDesigner2.0: A process diagram editor for gene-regulatory and biochemical networks.

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Systems biology is characterized by synergistic integration of theory, computational modeling, and experiment. Though software infrastructure is one of the most critical components of systems biology research, there has been no common infrastructure or standard to enable integration of computational resources. To solve this problem, the Systems Biology Markup Language (SBML) [1] and Systems Biology Workbench (SBW) [2] have been developed. A number of simulation and analysis software packages already support SBML and SBW, or are in the process to support it.
An identification of logic and dynamics of gene-regulatory and biochemical networks is a major challenge of systems biology. We believe that such network building tools and simulation environments using standardized technologies play an important role in software platform of systems biology. As one of the approaches, we have developed CellDesigner [3], which is a process diagram editor for gene-regulatory and biochemical networks.
The aim of the development of CellDesigner is to supply a process diagram editor with standardized technology for every computing platform so that it benefits the users as many as possible. By using the standardized technology, created model can be easily used with other applications which use standardized technology, thus it reduces efforts of users to create a model for each editing/simulation/analysis tools. The main features of standardized technology which CellDesigner supports are "Graphical representation", "Model description", and "Application integration environment".
CellDesigner supports biologist to easily create and simulate gene-regulatory and biochemical networks using solidly defined and comprehensive graphical representation [4]. CellDesigner is SBML compliant, and SBW-enabled software so that it can import/export SBML described models, and can integrate with other SBW-enabled simulation/analysis packages. CellDesigner runs on Windows, MacOS X, Linux and other UNIX platforms. The current release version of CellDesigner2.0 is freely available from http://www.celldesigner.org/.
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T-P08 Simulation of epidermal homeostasis including barrier formation

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Epithelial homeostasis is a complex self organizing system of special interest since 85% of cancers arise in this tissue. Although computational models should lead to a better understanding of the processes, there is, for example, still no model reproducing a horizontally layered epidermis emerging from a limited set of stem cells on an undulating basal membrane. We propose such a model integrating temporal, spatial and functional aspects. In principle, the model treats epidermal homeostasis as a steady granular flow and allows the free movement, division and removal of keratinocytes. Cycling stem cells generate cycling transit amplifying cells giving rise to keratinocytes committed to differentiation. Differentiation is dependent on extracellular calcium levels. Following the literature, our model has a transepidermal water flow transporting particles towards the surface and leading to particle loss. The epidermal barrier is formed by the secretion of lipids enclosed in lamella in deeper layers. An intact barrier traps calcium ions forming a calcium gradient. This gradient automatically results in a correct layering of differentiated cells. The model is implemented as an interactive simulation environment, which allows one to modify parameters and follow properties such as 2D morphology and particle flow. Charts show gradients, differentiation and kinetic parameters. Given the success modelling a small set of physical processes, we now intend to extend the methodology to include more effects and permit the study of other skin processes.

T-P09 Modelling protein motions for systems biology

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Structural biology provides atomic resolution descriptions of proteins and related biological molecules. To link structures to systems descriptions, methods for predicting the large-scale conformational dynamics of proteins are needed. Such methods will enable us to describe the dynamic properties of individual components (i.e. nodes) of complex biological networks.
In this study we have used the Gaussian Network model to define domains of a protein in relationship to their predicted movements, by analysis of the eigenvectors and cross correlation of motions over the whole protein. We have used this approach to examine protein components of complex membrane transport systems from bacteria. Twisting and fraying motions could be identified in barrel-like structures, including TolC, BtuB, and OmpA. Opening, twisting and rocking motions could be identified in clam-like periplasmic binding proteins such as BtuF and GluR0. In larger proteins, such as ABC transporters, the relationships between opposing transmembrane and nucleotide binding domains could be seen. In toroidal proteins, motions were identified showing that the domains could act in a fashion comparable to simpler ring structures such as sugars. GNM provides a valuable tool in identifying such motions and a strong starting point for linking systems biology with structural biology.

T-P10 Speeding up the central metabolism in *Pichia pastoris*

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Pichia pastoris is an excellent host for the production of heterologous proteins and has major advantages for the production of eukaryotic proteins when compared to the common host *Saccharomyces cerevisiae*. Especially its capability of introducing posttranslational modifications with similarity to that of higher eukaryotes, its rapid growth to high cell densities and its folding capacity for eukaryotic proteins makes *P. pastoris* well suited for the ever growing market of recombinant proteins.
From a metabolic point of view are the two yeasts well distinct. First, *P. pastoris* can utilize methanol as sole energy and carbon source. Second, the metabolism of *P. pastoris* under glucose excess conditions is highly respiratoric, thus this yeast is Crabtree negative. And third, the electron transport chain of *P. pastoris* is made up of two alternative oxidases. Therefore, in addition to its biotechnological importance, *P. pastoris* is an excellent model organism for eukaryotic energy metabolism.
We isolated and cloned the alternative oxidase (AOD) of *Pichia pastoris*. It enables adaptation to a wide variety of environmental constraints and has been found in every higher plant and most fungi tested. In yeasts occurrence of alternative oxidase is very common, but almost exclusively present in species known to be Crabtree negative.
Manipulating the electron transport chain in a respiratory yeast will directly influence the entire metabolism through the system-wide high integration of redox co-factors such as NAD(P)H and the energy currency ATP. Thus, respiration is an excellent target for research but should also be a major target for the engineering of industrial microorganisms.
We therefore analysed respiration modified *P. pastoris* strains under different defined environmental conditions using quantitative physiology and ^{13}C based metabolic flux analysis. This baseline study should end up in a metabolic model which will be very useful for the inclusion of more accurate physiological data into the modeling of fermentation processes.

T-P11 Software components for analysis of DNA microarray and quantitative proteomics data

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Although DNA microarray and LC-MS/MS with stable isotope labeling, such as SILAC (stable isotope labeling by amino acids in cell culture) and ICAT (isotope coded affinity tags) produce complementary results, they have not yet been integrated into one software package. As transcription and translation are mostly uncoupled processes in eukaryotes, a number of intervening steps, like selective mRNA degradation and stimuli-dependent translation can contribute to a big difference between mRNA and protein concentration in a single gene. Furthermore, the data on the dynamics of regulatory post-translational protein modifications (e.g. phosphorilation) can be combined with the mRNA profiling information to obtain the snapshot of entire signaling cascades. Our software components take into account conceptual similarities between ICAT- and SILAC-based quantitative proteomics and DNA micorarray data. They provide three types of modules: components for analysis of DNA microarray data, components for analysis of quantitative proteomics data and components for comparison of quantitative proteomics and DNA microarray data. All components are programmed in Java language with some data modules encoded in XML. Components for separate analysis of quantitative proteomics and DNA microarray data share a number of similarities, and both of them offer several tools and algorithms: importing of DNA micorarray or tandem-mass spectrometry data; basic statistical methods for comparison of quantitative proteomics or DNA microarray experiments; clustering and classification; visualization tools. The distinct feature of our software components are tools designed for direct comparison of DNA microarray and quantitative proteomics data. These tools include new data structures for storage of DNA microarray/quantitative proteomics data, modified algorithms for analysis of mRNA/proteins dynamics in time course experiments with time shift for uncoupled mRNA/protein expression, methods for mapping mRNA splice variants to corresponding protein isoforms based on their expression values. A number of image representation algorithms based on relevance networks are under development, which will allow mutual representation of DNA microarray/quantitative proteomics data. Bayesian networks for classification of clinical and other samples for DNA microarray/quantitative proteomics data are also being incorporated into software components.

T-P12 Systemic models for metabolic dynamics and regulation of gene expression – easy access, retrieval and search for publicly available gene expression data

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Studies of gene expression using microarrays result in huge amounts of data. Many scientific journals require data to be publicly available at the time of publication, but generally no specific requirements are made regarding what kind of data and in which format they must be submitted. The major repositories today are ArrayExpress (AE), GEO and SMD. The suggested standards like MIAME, and for presenting expression data in XML form, MAGE-ML are not in full use. Therefore, most sources offer the data in unique formats. The kind of data offered also varies from images and raw data to normalized log-ratios. In practice researchers need to search for, download and modify each dataset extensively before it can be utilized. This makes data retrieval, evaluation and comparison cumbersome. We believe that the images and the raw, untreated data should always be made available.

The aim of the project is to bring together data scattered in different sources for easy access, search and retrieval, and to offer this data in a suitable form for modelling and other data analysis purposes. The project is carried out in collaboration with two university groups modelling the yeast gene expression data. Therefore, our primary interest is in publicly available expression data for *Saccharomyces cerevisiae*.

The project is linked to a larger development plan for a systems biology platform at CSC. In a parallel project ongoing at CSC, a repository and an analysis environment is being built for microarray data. Our collection of yeast gene expression measurements will be added into that database. A comprehensive database will enable researchers to easily retrieve previous experiments for analysis, comparison with private data and modelling.

At present we are aware of 136 published datasets containing more than 4500 individual slides. Of these about 300 slides (20 publications) are only available on individual Internet pages. Only 8 publications can be found in AE, but AE contains more than half of the available slides. About 2000 slides are unique to AE, whereas SMD and GEO contain about 250 unique slides each.

The gene expression data will be linked with supporting information to enable retrieval of relevant data for model building. The system should enable extensive queries – e.g. data from cells growing under iron limitation, cells having a deletion in HXT1, and so on.

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T-P13 NMR spectroscopy in systems biology: methods for metabolomics and fluxomics

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Systems biology approach aims to finding links between different levels of cell function along with determination of links between components on a single level. Since all the levels interact constantly, the analysis of only transcriptome and proteome levels provides quite incomplete information of the system level function. NMR spectroscopy has numerous advantages in analyses of the metabolome and the fluxome, the complement of the metabolic fluxes.
The strength of NMR methods in metabolomics is their versatility. ^{1}H NMR is superior in being unbiased and more sophisticated methodology and detection of other nuclei can be used in targeted analyses. We are currently using NMR methods both in rapid metabolite profiling and in identification and quantification of the metabolites. Varian's cryogenic probe has provided a huge increase in sensitivity to the analyses. A spectra library that is being built will aid identification of the yeast metabolic intermediate signals. The metabolome profile NMR spectra are converted to multivariate data sets and multivariate data analyses such as principal component analysis (PCA) are being made.
Carbon-13 labelling experiments are currently the only means to obtain direct information on the metabolic fluxes in the system. One of the most effective carbon-13 tracer protocols is metabolic flux ratio (METAFoR) analysis (Szyperski *et al.*, 1999) where a fraction (10%) of uniformly labelled carbon source is used. Flux ratios can be determined measuring a single two-dimensional ^{1}H-^{13}C HSQC NMR spectrum of hydrolysed biomass. We have extended the method to the eukaryotic organism *Saccharomyces cerevisiae*, with compartmentalised metabolism, under glucose repressing conditions (Maaheimo *et al.*, 2001), and also included the glyoxylate shunt to the formalism. METAFoR analysis provides a global profile of the flux state of the system and the ratios can be used as constraints in metabolic flux analysis (MFA) (Fischer *et al.*, 2004). Experiments that employ positionally labelled carbon source molecules possess higher information content. A phosphorus-31 NMR based method, ^{1}H-^{31}P HSQC-TOCSY, for detection of positional fractional enrichments in sugar phosphate intermediates has been developed.
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T-P14 Autonomous oscillations in *Saccharomyces cerevisiae* during batch cultures on trehalose

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In a previous work, we have shown that exogenous trehalose assimilation in *Saccharomyces cerevisiae* is purely oxidative and takes place by two independent pathways (Jules, M, et al., 2004, AEM, 70, 2771). The main one relies on the periplasmic hydrolysis of trehalose by the acid trehalase Ath1p, while the second route requires the coupling of the trehalose uptake (Agt1p) and its intracellular hydrolysis (Nth1p).
Recently, we reported for the first time **autonomous oscillations in discontinuous cultures** (batch) of *S. cerevisiae* growing on trehalose as the sole carbon source. This unexpected oscillatory behaviour was examined from online gases measurements data (Mass Spectrometry) using **Fast Fourier Transformation** (**FFT**). This robust mathematical analysis, coupled to phase portrait diagrams, highlighted the existence of **two types of oscillations** as well as basic information about their properties. **The first type of oscillations was found to be linked to the cell cycle** since (i) the periods were fractions of the generation time, (ii) the oscillations were accompanied by transient increase in the budding index, mobilisation of storage carbohydrates and fermentative activity, and (iii) the occurrence of this type of oscillation was dependent on the specific growth rate (use of mutants exhibiting distinct, and constant or variable specific growth rates). **The second type are sustained, short-period, respiratory oscillations** and are independent of the specific growth rate. These two types of oscillations were remarkably identical to the cell cycle related and short-term oscillations that are usually observed in aerobic glucose-limited continuous cultures in the dilution rate range of 0.03 to 0.15 h-1 (Beuse, M, et al., 1998, J. Biotechnology, 61, 15; Lloyd, D, et al., 2003, FEMS Yeast Res., 3, 333). Another originality of this work was to find that contrary to previous considerations in continuous cultures, these two types of oscillations can take place consecutively and/or simultaneously during batch cultures on trehalose. In addition, mobilisation of **intracellular trehalose emerged as a key parameter for sustainability of ultradian oscillations** (subject of *in silico* investigations). Altogether, batch cultures on trehalose could be an excellent device to further investigate the molecular mechanisms that underlie autonomous oscillations (Jules, M, et al., 2004, Eur. J. of Biochem., submitted).

T-P15 Automated construction of genetic networks from mutant data

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Geneticists often use mutations to investigate biological phenomena [2]. Mutations cause changes of organism's phenotype and may reveal which genes participate in a certain biological process and how. To represent these functional interactions between genes, a genetic regulatory networks are an often used formalism [1].
We have developed a system called GenePath for automated construction of genetic regulatory networks from mutant data. GenePath considers classical genetic data where a phenotype is observed for a set of single or double mutations. Prior knowledge, expressed through relations between genes (possibly extracted from the relevant literature) can also be included. GenePath employs a set of logical patterns of the type “IF there exist a set of experiments that involves genes A, B, ... THEN a certain relation between these genes can be inferred”. These relations are then used to propose genetic networks. An important feature of GenePath is the ability to explain each relation from the constructed network by reporting on the logic that was used to infer it together with the corresponding experiments.
GenePath thus formalizes genetic data analysis [3], facilitates the consideration of all the available data in a consistent manner, and allows for the examination of the large number of possible consequences of planned experiments. We will illustrate through an example the advantage of using an automated approach and report on recent extensions of GenePath that include (1) handling of uncertainties in genetic data by allowing to assign confidence to experiments and background knowledge, (2) assistance in experiment planning by proposing a set of the “cheapest” experiments to assert new gene-to-gene relations, (3) interactive what-if analysis, which enables the user to on-the-fly test alternative hypothesis about the organism's regulatory mechanisms, and (4) handling of cyclic pathways through detection of genes that are involved in such network and appropriate visualizations. GenePath is implemented as a web-based application that is available at <Ahref="http://www.genepath.org" target="_blank">http://www.genepath.org. References: (1) Altman, R.B. et al., (2001), Curr Opin Struct Biol., 11(3), 340. (2). Avery, L. et al., (1992), Trends Genet., 8(9), 312. (3). Zupan, B. et al., (2003), Bioinformatics, 19(3), 383.

T-P16 An integrative framework for modeling signaling pathways

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Objective: Much of the effort to understand signal transduction has been aimed at identifying the molecules that participate in signaling cascades and at qualitatively characterizing the activities and interactions of these molecules. Improving our understanding of pathways typically involves an iterative interplay between experimental and modeling strategies. The intention of the proposed framework is to enable such an iterative interplay. The formation of protein complexes based on protein-protein interactions was investigated and used to define the requirements for that framework.
Methods: The framework includes environments for pathway visualization and simulation, as well as a knowledgebase providing biological data (components and interactions), modeling data (chemical-reaction network of the components and its rate constants), simulation data (sensitivity analysis), and experimental data (connectivity map of the components and RNA interference experiments). The formation of protein complexes was investigated using a recently produced model of the TNFalpha-mediated NF-kappaB pathway [1]. An integrated approach of proteomic pathway mapping based on established components provided experimental information [2].
Results: Modeling, simulation, and sensitivity analysis with respect to rate constants and initial concentrations were performed. Modified mathematical models with new added proteins and modified protein-complexes were reconstructed based on given experimental data. Experimental data of systematic single gene expression perturbations using RNA interference, which in some respects mimics pharmacological treatment, were used to validate the behavior of the produced mathematical models.
Conclusion: The proposed framework might allow one to simulate experimentally observed input-output relationships of signaling pathways and might support the iterative interplay between experimental and modeling strategies. In addition, the efforts of modelers and quantitative experimentalists will have to be tightly integrated in order to understand how the components in a signaling cascade work in concert.
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T-P17 Data visualization for gene selection and modeling in cancer bioinformatics

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Microarray technology enables simultaneous investigation of expression of thousands of genes and has become a major tool for genomic studies of human cancer. Gene expression studies may contribute to more effective tumor classification and provide insights into pathogenesis, diagnosis, prognosis, therapeutic targets and clinical outcome of tumors. While the goal of such studies - finding genes that can differentiate between groups of outcomes - is clear, the analysis of experimental data sets is complex and difficult. Modeling of such data belongs to the domain of supervised learning methods. Such methods that are most often applied in cancer bioinformatics include support vector machines and artificial neural networks. Although these methods may find accurate predictive models, their interpretation is difficult and thus their contribution in discovery of new knowledge rather limited. In contrast to complex computational models (1), we claim that simple data visualization techniques may be used to clearly expose discrimination between different types of tumors.

In research reported here we used the data sets from the Cancer Program of the Broad Institute (http://www.broad.mit.edu/cgi-bin/cancer/datasets.cgi). We evaluated the predictive power of expression of genes using a standard, well-known non-myopic measure called ReliefF. Our experiments show that in most cancer microarray data sets, there are at most few tens of genes which have distinctively highest predictive value. We have also observed that their functional annotation related them to the biological processes in observed cancers.

To search for visualizations where data points belonging to distinct outcomes are well separated we used a method called VizRank. VizRank is able to automatically rank visual projections according to estimated degree of separation of outcomes, and provides heuristics to search only through the space of most promising visualizations (2). To visualize the expression data, we used either scatterplot or radviz. For most of the data sets, VizRank found radviz visualizations where separation of groups with different outcomes was clear and obvious. This method provides for a simple, understandable, and significatly less sophisticated classification model than prevailing techniques used in current cancer bioinformatics practice.

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T-P18 Accelerating the construction of genome-scale metabolic models: a test case for *Lactococcus lactis*

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In the past few years, a large number of sequenced and annotated genomes have become available and many more genome-sequencing projects are still in progress. The availability of the genomic information of a species allows genome-scale metabolic reconstruction based on its annotation and on existing metabolic information from other species.

The process of metabolic reconstruction is extremely time-consuming. We have developed (and benchmarked for *Lactoccocus lactis* IL403) a method to benefit from available well-curated metabolic databases to accelerate this time-consuming process. First we created an in-house database of orthologous genes for a selected number of species. The procedure for constructing the database of orthologous genes is based on a combination of COG [1] and INPARANOID [2], resulting in an improved orthology definition. Then we selected orthologous hits with genes of *L. lactis* to retrieve metabolic information from manually curated metabolic databases of *E. coli*, *L. plantarum* and *B. subtilis*. We used both public data from EcoCyc (http://ecocyc.org/) and LacplantCyc (http://www.lacplantcyc.nl/) and proprietary data from SimPheny models (Genomatica Inc.).

The number of automatically assigned gene-reaction associations, using Pathologic software [3] and Genbank information for *L.lactis* (2400 genes), was 483. With the described method we identified 207 extra putative gene-reaction associations. Using SimPheny models of *E. coli*, *L. plantarum* and *B. subtilis* we were able to assign 336 likely and 183 putative gene-reaction associations for the construction of a *L. lactis* SimPheny model. The total assignment of gene-reaction associations by manual curation is still in progress.

The results show that we can accelerate the metabolic reconstruction by taking optimal advantage of already existing, manually curated databases and models.

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T-P19 Application of yeast genomic strategies to link biologically active compounds to their cellular targets

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Bioactive compounds can be valuable research tools and drug leads, but it is often difficult to identify their mechanism of action or cellular target. To address this, we have recently developed a genomic platform in the budding yeast *Saccharomyces cerevisiae* that we believe is an effective and broadly applicable method for deciphering the cellular pathways and proteins affected by inhibitory compounds (Parsons et al., (2004) Nat Biotechnol, 22:62-9). Here, comparing chemical-genetic interaction profiles, where the complete set of yeast viable mutants are tested for hypersensitivity to a target-specific compound, with a compendium of global genetic interaction profiles provides a powerful key for deciphering the pathways and targets of the growth-inhibitory compounds. In concert with chemical-genetic profiling we are currently employing additional yeast genomic tools such as expression profiling, drug induced haploinsufficiency analysis, and mapping of drug-resistant mutants using Synthetic Genetic Array (SGA) methodology to identify targets of novel anti-fungal compounds derived from natural product extracts. In particular, Papuamide B (Pap B), a high molecular weight cyclic lipopeptide, is intriguing because our analysis suggests that it may function similarly to Caspofungin, an echinocandin-like cyclic lipopeptide that disrupts the yeast cell wall by inhibiting 1,3 beta-glucan synthesis. The chemical-genetic interaction profiles for Pap B and Caspofungin are highly similar, containing numerous common genes, many of which are associated with in cell wall organization and biogenesis, cytokinesis, and chitin metabolism. However, unlike Caspofungin, Pap B does not inhibit beta-1,3-glucan synthesis *in vitro*, suggesting it functions through a different but functionally related target. To gain further insight into the mode of action of Pap B, we are applying our SGA mapping methodology to identify Pap B resistant mutants. We have identified one Pap B resistant strain as a *cho1* mutant. *CHO1* encodes a non-essential enzyme required for the synthesis of phosphatidylserine (PS), one of four major phospholipids found in yeast cell membranes. Further analysis is suggestive of an interaction between Pap B and PS and we are currently examining whether Pap B may be entering the cell via PS and then acting on an intracellular target or if Pap B could be exerting its effect directly through PS.

T-P20 SCIpath - an integrated environment for systems biology analysis and visualisation

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The SCIpath toolkit is a SBML-aware suite of analysis programs for microarray and proteomics data analysis that is open source and programmed exclusively in Java. SCIpath provides a flexible and extensible environment for the interpretation of functional genomics data through visualisation. The first version of the application (downloadable from the SCIpath website), implements four tools that include functionalities such as editing and visualisation of custom-made pathways, visualisation and manipulation of microarray data alongside pathway data and a fuzzy clustering algorithm for gene expression data. The current tools provide a simple yet powerful way of graphically relating large quantities of expression data from multiple experiments to cellular pathways and biological processes in a statistically meaningful way. SCIpath is an expandable toolkit with the aim of promoting understanding of systems biology by bringing together different aspects of cellular biology through visualisation and analytical techniques. This objective is realised through the integration of applications, written independently by other programmers, in a plug-in style interface that enables the end-user to manipulate different types of data from different applications to build innovative visualisations and analytical software components. By using a structured framework that controls the messaging between applications in a service-oriented fashion, the SCIpath design enables the user and programmer to port the functionalities of different applications so that biological data from multiple applications can be visualised intuitively, thereby enhancing the understanding of the biological system.

T-P22 Glycolytic oscillations in spatially ordered interacting cells

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Synchronisation of glycolytic oscillations in populations of yeast cells have been intensively analysed experimentally [1] as well as theoretically [2]. There is evidence that the individual cells communicate by exchanging products of glycolysis as acetaldehyde, for which the plasma membrane is permeable. There are, however, a number of discrepancies between theoretical predictions and experimental results. For example, in existing models synchronisation occurs much slower than observed in populations of yeast cells [2]. In extension to previous models which considered stirred cell suspensions we study oscillations in spatially ordered cells. We aim to reproduce data of experiments where glucose is added to starved cells in a limited region of the cell layer initiating in this way a wave resulting from the propagation of glycolytic oscillations [3]. For the generation of oscillations in the individual cells a simple model of glycolysis is used containing an autocatalytic step. Cells are embedded in an extracellular medium in which the added glucose and the extracellular product can diffuse. The model takes into account special kinetic properties of glucose carriers in yeast cells. Intercellular coupling takes place via diffusion of the end product between neighbouring cells. For a small number of linearly ordered cells, the oscillations can be studied by using bifurcation analysis. We could find very complex oscillatory states already for three interacting cells with a uniform glucose input. The model is extended by considering linear arrangements of many interacting cells. Moreover, we studied regular and irregular two-dimensional spatial arrangements. When glucose injection is confined to a limited number of neighbouring cells, we observe the formation of propagating waves of glycolytic oscillations over the whole arrangements. The characteristics of wave formation are studied in dependence on the diffusion of glucose in the extracellular medium, the uptake of glucose by the individual cells, as well as on the permeability of the membrane for the coupling substance. It is shown that the existence of waves depends crucially on the strength of the coupling.

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T-P23 Database Support for Yeast Metabolomics Data Management

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The enormous flood of omics data brings the need for well-designed and -curated databases, which can store, handle and disseminate large amounts of data efficiently and readily lend themselves to data mining techniques used to extract hidden patterns from the data. The extracted facts can be further explored in simulation-based analyses providing predictions to be tested in vivo/vitro. Such a framework consisting of modelling, machine learning and simulation can help achieve the systems biology objective (to understand the way in which the heterogeneous parts of biological systems combine to form the whole).
The first step is to develop and standardise the omics models and populate them with curated data. The proteomics field has significantly advanced in such efforts: HUPO has driven the Protein Standards Initiative in order to develop an exchange standard for proteomics data (MIAPE) based on the PEDRo schema. Similarly, MAGE-ML represents an emerging standard for transcriptomics. Some attempts have been made in metabolomics, e.g. in ArMet, a data model for plant metabolomics (www.armet.org).
We developed a similar approach for yeast metabolomics with an emphasis on metabolomic footprinting as a strategy for functional genomics. Nonetheless, the general schema is applicable to a wide range of metabolomics experiments. The core schema (modelled in UML) consists of abstract classes, which can be specialised in order to embrace different types of experiments, results, organisms, etc. It is designed to capture information about the overall experimental cycle, including growth, sample preparation and analytical experiments. Storage of the information about specific conditions, protocols and parameters used in wet experiments (i.e. the metadata) is needed to interpret the experimental results and support their comparison and reproducibility. In addition, metabolomics experiments in the post-genomic era often need to be extended beyond the traditional wet experimental framework. In order to process the vast amount of metabolomics data, data mining experiments (or dry experiments) need to be performed *in silico* to extract knowledge.
Our metabolomics database model has been implemented as a relational database and an XML schema. In both cases, flexibility has been supported by using a modular approach where different metadata modules (implemented as separate XML schemas) can be plugged into the overall metabolomics schema (in both relational and XML versions).

T-P24 Fokker-Planck equations for IP_3 mediated Calcium dynamics

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The stochastic behavior of release channels on the membrane of the endoplasmic reticulum has proven essential for intracellular Ca^{2+} dynamics. This holds in particular for inositol-1,4,5-trisphosphate (IP_3) mediated Ca^{2+} release through IP_3 receptor channel clusters. Recent studies have revealed two states with an increased probability. In a deterministic model, these states correspond to stable stationary states. While one is a state of low Ca^{2+} concentration with no channels open, the second represents a state with high Ca^{2+} concentration known as a Ca^{2+} puff. The impact of fluctuations in such a system is reflected in transitions from one stationary state to the other. Puffs are pivotal for the intracellular Ca^{2+} dynamics because a concerted action of several puffs can initiate vital Ca^{2+} waves. Therefore, we derive a master equation and corresponding Fokker-Planck equations for the stochastic dynamics of an IP_3 receptor channel cluster. It incorporates the strong localization of the channel clusters on the membrane of the endoplasmic reticulum and the ensuing large Ca^{2+} concentrations at an open cluster. We employ our ansatz to compute the stochastic fraction of the puff frequency. Our results shed new light on the stochastic aspect of puff occurrence.

T-P25 Global transcriptional response of *Saccharomyces cerevisiae* to ammonium, alanine, or glutamine limitation

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Cell physiology and changes on the gene expression level under nitrogen limitation of the haploid yeast *Saccharomyces cerevisiae* strain CEN.PK-113-7D were studied using nitrogen limited chemostat cultures and global transcription analysis tools. S. cerevisiae was grown separately at alanine, ammonium, and glutamine limitation in aerobic chemostat cultures with a dilution rate (D) of 0.2 h^{-1}. In order to make nitrogen limitation studies more comprehensive, transcription data was analyzed together with results obtained from glucose limitation studies. Genome scale metabolic model knowledge and various bioinformatics tools were applied in the transcription data analysis in order to study cell physiological response to the specified limitations. Results showed that central nitrogen metabolism was affected not only due to nitrogen limitation, but it also depended on the limited nitrogen source. Genes with altered expression level under every nitrogen limitation were involved in amino acid metabolism, protein biosynthesis, cell organization, ion homeostasis, transcription regulation, energy production and various transport systems. Alanine limitation produced the most drastic differences on the yeast metabolism. Group of genes involved in nitrogen catabolic repression, amine group, or pyruvate metabolism had changed expression, including strongly affected alanine aminotransferases coding genes *YDR111C* and *YLR089C*. Transcription analysis results were consistent with physiological observations and indicated that yeast's ability to grow under various nitrogen limitations was acquired by altering regulation of central nitrogen metabolism and through propagated changes in other metabolic pathways, crucial to cell growth and development.

T-P26 Identification of the C-terminal signal peptides for GPI modification and prediction of the cleavage sites.

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Glycosylphosphatidylinositol (GPI)-anchored proteins represent a subclass of cell surface proteins found in all eukaryotic cells. Knowledge of a protein's GPI modification is very valuable, since it defines its subcellular localization and limits the range of possible cellular functions. The modification takes place in the Endoplasmatic Reticulum (ER) and involves enzymatic removal of a C-terminal signal peptide (much like N-terminal signal peptides), followed by addition of the sugar moeity to the new C-terminal amino acid residue of the protein.
A number of sequence based methods have been developed for the prediction of GPI-anchored proteins, most of which rely on defining a C-terminal consensus sequence for GPI modification. However, only about 40 proteins with experimentally verified cleavage sites are known to date. Many of the prediction tools currently available are therefore based on protein examples for which the GPI signal sequence and the cleavage site are either inferred from homology or based on predictions by previous *in silico* methods.
Here we present a new sequence based prediction tool of GPI-anchored proteins, based exclusively on experimentally verified data extracted from Swiss-Prot. The method is based on artificial neural networks - a machine-learning method able to capture non-linear context dependent patterns. The predictive performance of the method is better than all other methods currently available.

T-P27 The *Genevestigator* gene function discovery engine

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Despite its short history, the era of gene expression profiling has already acquired an expertise that matches up to the virtue of many older sciences. From the beginning, hopes to rapidly discover the function of genes fostered the development of ever better analysis tools. Nevertheless, progress in computational tools to rapidly identify gene function is still facing a prevailing gap between wet lab biologists and the bioinformatics world. Moreover, the genome-wide study of gene expression in higher organisms has furthermore increased the level of complexity, outstripping the ability of many scientists to analyze and interpret this bulk of data. This discrepancy challenges the scientific community to increase the pace of developing computational analysis capabilities targeted to non-bioinformatics users.
Epitomizing the gap between data generation and data analysis, the availability of tools for biologists to easily query large microarray datasets to retreive the properties of genes of interest is currently scarce. Genevestigator [1] is an Arabidopsis microarray database with a suite of tools for gene function discovery. User-friendly web-based query forms allow to retrieve the expression patterns of genes of interest in chosen contexts, such as plant development, organs, and responses to stresses or mutations. Further tools allow to indentify marker genes expressed specifically in these categories or responding to particular conditions. The Genevestigator suite of tools is being completed by a metabolic and regulatory pathway map that will allow users to match genes of interest to these pathways and to identify genes involved in different biological processes. The goal of Genevestigator is to drive lab research by providing contextual information about the expression of genes, allowing targeted design of new experiments.
As of December 2004, 1500 plant scientists have registered and more than 50 distinct users query the database daily. Owing to the strong interest from other communities, we look for partners to help extend Genevestigator to other organisms. In fact, a multi-organism and comparative genomics platform could further contribute to fulfilling the hope for an accelerated discovery of gene function.

1. Zimmermann et al., 2004. Plant Physiol 136: 2621-2632.

Power posters

T-PoP1 1/f Noise in Ion Channel: A Theory Based on Self-Organised Criticality

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The aim of this work is to investigate the noise profile of Voltage Dependent Anion Channel (VDAC). VDAC from mitochondria of rat brain was isolated and purified using standard procedure, and reconstituted into a planar lipid bilayer (Banerjee & Ghosh, 2004). Single-channel currents were recorded under different voltage clamp conditions across the membrane. Power Spectrum analyses of the current-time traces were done. It was found by the slope measurement of the power spectrum (spectral density versus frequency log-log plot) that the open state noise of single-channel VDAC follows power law and the noise is of 1/f nature. Moreover, this 1/f nature of the open channel noise is maintained throughout the range of electric potential -45 to +45mV. Having obtained this result on open channel noise we looked into the origin of the 1/f pattern in ion channels. It is interesting to note that 1/f noise has been observed in a wide variety of systems ranging from earthquake, turbulence to electrical circuits and now in biological membrane system. We have used the concept of Self-Organised Criticality (SOC) and proposed a model to explain the existence of power law (1/f) in channel noise. It is being proposed that in this process of passive diffusion transport through VDAC, the 1/f noise arises out of defects in the passage of ions across the membrane. In doing so an analogy has been drawn between the sand pile avalanches (Bak et al, 1987) and the sudden opening of the VDAC. Although the theory proposed by us is based on the experimental data (bilayer electrophysiology) on VDAC, it should hold for ion channels in general.
Reference:

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T-PoP2 Single cell mechanics and mechano signal transduction using a micro-force loading device

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The interplay between molecular forces within a biomolecule, between biomolecules and sub-cellular structures and biochemical processes is vital in maintaining proper functioning of a living system. However, how exactly the molecular machines of a living system are organized, regulated, and challenged by various forces are not fully understood at the present. A general strategy to reach such an understanding is to disturb the otherwise dynamically equilibrium living system by externally applied force and study its effects on various levels of cellular structures.
In this study we have developed an integrated analytical system for studying cellular mechanics, in which a loading device with a force resolution of 0.01 micro newton and displacement resolution of 1 nm constructed (Fig. 1). The loading device is in assembly with a confocal force microscope such that mechano-signal transduction at the micrometer scale (through real-time imaging of deformation of a single cell, Fig. 1) and at the nanometer scale (through real time visualization of the deformation of green fluorescence protein labeled cytoskeleton), can be quantitatively interpreted. At the cellular level, interpretations of experimental data were also carried out by using continuum mechanics modeling (finite element method, Fig. 2) of a single cell, and at the molecular level, using molecular dynamics simulation (Fig. 3).
Applying the novel nano-analytical tools coupled with modeling at multiple length scales for studying the complex mechano-biochemical-driven cellular processes at the molecular/cellular level will provide new systematic insights into the physical principles of mechanics-driven functions, e.g., injury, organogenesis, etc, and powerful design principles for novel diagnostic and therapeutic strategies.

T-PoP3 Connectivity matrix for describing all the atom-level connectivities in a given metabolic network and its use for analysis of the network structure

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Atom is the smallest node in metabolic networks. Therefore, atom-level consideration is important in the understanding of metabolic networks. Atom-level connectivity in metabolic networks is classified into inter-metabolite connectivity through enzymatic reactions and intra-metabolite connectivity through chemical bonds. Evolutional co-emergence of a new metabolite and a new enzymatic reaction responsible for the metabolite is a reasonable idea, suggesting potential correlation and probably complicated relationship between inter- and intra-metabolite connectivities in the network structure. On the other hand, membrane transport is common biological phenomena and stoichiometry is one of the most important characteristics in metabolic networks. Here, I present a data format for describing both inter- and intra-metabolite atom-level connectivities including information of compartmentation and stoichiometry. In the present format, each atom is expressed as a row vector composed of 4 integers indicating metabolite species, position of atom in the metabolite, atom species, and compartmentation. Information about connectivity itself is also expressed as a row vector composed of 3 integers. A vector for inter-metabolite connectivity includes 1 (connectivity-type number), reaction number, and stoichiometry number which indicates how many times the connectivity appears in the reaction, whereas a vector for intra-metabolite connectivity includes 0 (connectivity-type number) as the 1st component, and bond-type number as the 2nd component. Thus, conversion of atom va1 to atom va2 through reaction vr is expressed as (va1, va2, vr), a row vector formed by the combination of 3 row vectors. Using this format, all the atom-level connectivities in a network are expressed as a matrix, connectivity matrix (CM), each row of which corresponds to one connectivity. Using a database, IMAC (www.metabo-info.org), CM for a given network can be prepared. Structural analyses of the network can be performed on GNU Octave using the CM obtained and m-files for Octave scripts or functions. At present, calculation of the paths between 2 specific atoms and calculation of net balance of the reaction sequence constituting such a path can be performed using m-files for Octave. The present format is expected to contribute to the understanding of structure-function relationship of metabolic networks.

T-PoP4 Using SRS to develop and populate an information layer for the EMI-CD modeling platform

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The European Modeling Initiative Combating Complex Diseases consortium (EMI-CD; http://www.molgen.mpg.de/~EMI-CD/) aims to develop a modeling and data integration platform for complex and heterogeneous data sources, that can generate hypotheses and models for disease processes based on *in silico* predictions. LION (http://www.lionbioscience.com/) is developing software to construct and automatically populate an information layer, supplying exhaustive knowledge of the relevant biological objects (genes, proteins, protein complexes, organelles, cells, *etc.*). The information layer is based on the SRS data integration platform (Etzold, T., *et al.*, in "Bioinformatics: Managing Scientific Data", Lacroix, Z. and Critchlow, T. (eds), Morgan Kaufman, 2003), which can integrate data from diverse sources. Interaction with the analysis and modeling layers of the EMI-CD platform will use the SRS loader technology combined with a web services-based interface to provide the required data in an accessible, coherent and flexible manner.

A central source of data for this project is Reactome (http://www.reactome.org/), which has been incorporated into SRS, but additional sources include sequence-based databases (*e.g.*, RefSeq, Ensembl), structural databases (*e.g.*, PDB, CATH), model-based databases (*e.g.*, KEGG) and knowledge-based databases (*e.g.*, GeneCards). Further data sources will be added as the project progresses. Cross-reference and related information between these data sources will also be used to generate a network of information sources. In order to support specific simulation experiments, mechanisms using the original sources will be developed to generate data sets relevant to specific diseases, or disease areas.

It is of central importance to the information layer that the data is up-to-date and of high quality and internal consistency. An automated mechanism, based on SRS Prisma, will be developed to carry out automatic updating of data from remote repositories, further data processing, and quality assurance using tailor-made tests to check the quality of any new data.

The EMI-CD consortium consists of three academic partners and two SMEs, and is funded by the European Commission within its FP6 Programme, under the thematic area "Life sciences, genomics and biotechnology for health", contract number LHSG-CT-2003-503269.

Unicellular Organisms

Posters

U-S01 Modelling fission yeast morphogenesis

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Because of its regular shape, fission yeast is becoming an increasingly important organism to study cellular morphogenesis. Genetic studies have identified a great number of proteins that are important to regulate shape changes during the cell cycle. Most of these proteins interact with either microtubules or actin, underlining the essential roles these cytoskeletal structures play in cellular morphogenesis. Here we present a simple model for fission yeast morphogenesis that describes the interplay between these cytoskeletal elements. An essential assumption of the model is that actin polymerization is a self-reinforcing process: filamentous-actin promotes its own formation from globular-actin subunits via regulatory molecules. Microtubules stimulate actin polymerization by delivering a component of the autocatalytic actin-assembly feedback loop. We show that the model captures all the characteristic features of polarized growth in fission yeast during normal mitotic cycles. We show that all the major classes of morphogenetic mutants (monoipolar, orb and tea) are natural outcomes of the model. We categorize the types of growth patterns that can exist in our model and compared them with experimental observations.

U-S02 Metabolic quorum sensing: onset of density-dependent oscillations

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Populations of oscillating units coupled by diffusion through a homogeneous medium are studied as a model for cells in a CSTR. In particular, we focus on the dependence of the collective behaviour on the density of the cell suspension. Both the classical Kuramoto model and the recent results on "coupling by quorum sensing" (Garcia-Ojalvo, Elowitz and Strogatz (2004) PNAS 101,10955) indicate that, by diluting the suspension, the cells should keep their oscillatory behaviour while desynchronising.

A different scenario could however take place due to the delay introduced in the coupling by the presence of a medium. In this case, the dilution of the suspension results into the suppression of oscillations at both population and individual levels. Such density-dependent phenomenon may be seen as a metabolic analogous of quorum sensing in bacteria: the amplitudes of the individual metabolic oscillations can provide each individual cell with information on the population density and average state of the population.

U-S03 Integration of software tools for the *in silico* design of metabolic pathways using flux balance analysis

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The systems biology approach, where one can envision the cell as a whole is a step in the direction of narrowing the gap between the rate of data generation and the speed of analysis. This embraces the much desired goal of understanding the role of the metabolic pathways of a determined metabolic network.
This study describes the use of several software tools, integrated to perform the simulation of a specific metabolic pathway by Flux Balance Analysis (FBA). This simulator receives as input the stoichiometry, the thermodynamic and capacity constraints of the metabolic network, and also an objective function. The stoichiometry and the thermodynamic constraints are represented in the SBML format (Systems Biology Markup Language), whereas all the other information is represented in a plain text file. The SBML file is parsed using libSBML, which is a library that can be embedded into an application to read, write and manipulate files in the SBML format. The text file is parsed using FLEX, a lexical analyser that generates a C/C++ program that recognizes specific lexical patterns in the text. With this information, one can construct a Linear Programming (LP) problem. To solve it, GNU lp-solve is used. It uses the simplex algorithm and sparse matrix methods for simple LP problems. The solution provided is a possible flux distribution on the network, that maximises the objective function. In this work, data from a batch fermentation process (where the host system is *Escherichia coli* strain BL21) is incorporated in the model definition. In the experiment, Acetate secretion, Oxygen Uptake Rate (OUR), Carbon Evolution Rate (CER) and Biomass production for wild type (wt) and mutant (mt) strains were determined. The mutant was engineered with a plasmid to express the human recombinant interleukin 4(IL-4) using pRT as a promotor.
The analysis of the flux distribution for wt and mt is performed for the maximisation of ATP production, incorporating as capacity constraints the different data obtained from the experiment. A discrete time analysis was performed, using the same variables, and assuming a steady state for each time sample. Comparing FBA results for wt and mt, the induction of protein in the host system decreases the capacity of producing ATP. The sensitivity of the system to variations in glucose uptake was also performed. It was shown that, in normal conditions, mt and wt were robust to variations with an amplitude of 2% and 20%. When the carbon source is residual, the system shows a higher sensitivity to glucose variations.

U-S04 Uncovering the control of the respiratory clock in yeast

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Continuously growing yeast cultures tend to auto-synchronise producing a robust respiratory oscillation (tau circa 40 min). Recently we have carried out Affymetrix analyses that revealed the majority (>90%) of the transcriptome oscillates within this timeframe [1]. Here we analyse this data using a “Fourier focussing” technique in order to derive transcripts that are closely coupled to the oscillation. The method involved dividing the amplitude calculated by fast Fourier transformation by the mean of the amplitude for three oscillation cycles. This ratio equated to the noise of the transcript’s oscillation; where a perfect sine-wave generates a ratio of one and random data generates a ratio approaching zero. When the ratio was calculated for the yeast transcriptome and plotted, the resulting curve showed two gradients. The intercept of these gradients was used as a noise threshold (ratio of ~0.15; ~1500 genes). The strongly coupled transcripts above this threshold and phenotypic events were then used to construct a “clock face”. A network diagram was then constructed using high quality BIND and transcriptional regulatory networks within Cytoscape [2]. The resulting network consisted of ~1000 transcripts containing the most highly conserved aspects of the eukaryotic process, e.g., ribosome, proteasome, DNA synthesis, autophagy, cyclins, amino acid biosynthesis, carbon metabolism, stress response, respiration, etc. Furthermore two transcriptional sub-graphs out of phase with each other were identified. CIN5, YAP6, YAP1, PHD1 and ROX1 comprised the core of the sub-graph whose transcripts peaked during the low respiratory phase and MET4 and RAD59 comprised the sub-graph whose transcripts peaked during the high respiration phase. The cultures synchronisation mechanism revolves around the production of acetaldehyde and hydrogen sulphide [3], which feed into and out of this network via ALD5/ADH2 and SUL2/MET3 respectively. It is concluded that these networks regulate the respiratory clock within yeast. It is also postulated that this network may form the centre of an energetic “bowtie” common to all eukaryotes because of its high conservation among all eukaryotes.
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U-P01 Neisserial DNA uptake sequences: biased distribution and influence on transformation

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DNA uptake sequences (DUS) are short oligomers that occur in exceptionally high numbers throughout the genomes of, among others, *Neisseria* and *Haemophilus influenzae*. DUS are required for efficient transformation of DNA in these naturally competent bacterial species [1].

DUS are present both outside and within annotated coding regions. A previous report has shown that DUS were the most abundant 9-10 mers within coding regions in the genomes of the human pathogens *N. meningitidis*, *N. gonorrhoeae* and *H. influenzae* [2]. More importantly, a significantly higher density of DUS was found within genes involved in DNA repair, recombination, restriction-modification and replication than in any other group of genes. DUS in genome maintenance genes might provide a mechanism for facilitated recovery from DNA damage after genotoxic stress.

DUS outside genes very frequently occur as inverted repeats and are likely to be involved in transcriptional termination1. In order to fully determine the extent of inverted DUS repeats, all the DUS of the *N. meningitidis* MC58 genome were collected and investigated. Furthermore, a genome scanner for terminators was applied to identify intrinsic terminators. The two sets of data show that about half the *N. meningitidis* genes were terminated intrinsically and that half of these terminators contain DUS, making up a number of DUS that amounts to about half of the total number of DUS in the *N. meningitidis* genome. In summary, DUS seems to hold dual functions: mediating binding and uptake of transforming DNA with a bias towards genome maintenance genes as well as being an element in transcriptional termination. Currently, the biased distribution of intergenic DUS as well as the functional role of DUS in the transformation process are being assessed.

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U-P02 Gene expression and adaptive responses of *in situ* fermentation

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Lactic acid bacteria (LAB) belong to the most important microorganisms used in food fermentation. In recent years several genomes of LAB have been sequenced and the big task ahead in the post-genomic era is to elucidate the function, responses and interaction of genes and their products under various environmental conditions. *Lactococcus lactis* is an important component in dairy starter cultures and is one of the best characterised LAB species. Numerous studies carried out under laboratory conditions have resulted in detailed knowledge on the physiology and molecular biology of *L. lactis*. Yet, little is known about its response to complex environments during *in situ* fermentation. Recombinant *in vivo* expression technology (RIVET) allows studying the genomic response of an organism *in situ*(1). We will apply RIVET to *L. lactis* which will help to increase our understanding of the genomic response during e.g. the ripening of cheese. Currently adaptions are applied to the RIVET tools which will allow the high throughput screening of obtained target sequences under various conditions *in vitro*. This will lead to large functional datasets which will contribute to the further understanding of the cellular processes and responses of lactic acid bacteria. Eventually knockout and/or overexpression studies of relevant targets will allow their further evaluation.

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U-P03 Metabolic functions of duplicate genes in *Saccharomyces cerevisiae*

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The role of duplicate genes in *Saccharomyces cerevisiae* is still not known, despite exhaustive genome data of hemiascomycetous yeast nowadays available. In contrast to previous works that favored isolated functions we here propose a dispersed array of functions for the occurrence of duplicate genes in the yeast metabolism. These include back-up activity, hence complementation of a genetic dysfunction by a duplicate gene, regulatory function, enhanced enzyme synthesis by parallel transcription on multiple gene copies and a specialized role of enzymes within a duplicate family. The various functions were analyzed with genome scale phenotype testing and C^{13} constraint based flux analyses on 5 different growth conditions. In a highly integrative approach computational modeling was used to overcome the lack of an exhaustive library of complete duplicate gene family mutants. For our analysis we used a reconciled full genome scale metabolic model that was validated with in vivo data for the case of singleton gene mutants. It was such possible to predict the phenotype for the deletion of complete duplicate gene families and to analyze the inherent network topology.
Interestingly, for a significant number of duplicate genes no particular function could be attributed and phylogenetic clustering suggests that a fraction of these are “snapshots in time” and might be lost during evolution. This study, for the first time, quantifies the role of duplicate genes in the yeast metabolism and highlights that duplicate genes cover a wide spectrum of functions, which may explain the high number of duplicate genes present in *S. cerevisiae*.
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U-P04 Experimental manipulation and mathematical modeling of arginine biosynthesis in *Escherichia coli*

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The different elements of the arginine pathway and the principal regulatory mechanisms operating at genetic and enzymatic levels in E.coli are identified, allowing to integrate them in an initial mathematical model, using the XPPAUT software. The first enzyme of the pathway is N-acetylglutamate synthase, (NAGS) which acetylates L-glutamate using Acetyl-coA. NAGS is genetically repressed by the arginine repressor *argR* in the presence of arginine and feedback-inhibited by arginine. Carbamyl phosphate synthase (CPS) is at a branch point between the pryrimidine and arginine biosynthetic pathways: it provides carbamyl phosphate (CP) for both. It is subject to a cumulative genetic repression by arginine and pyrimidines and its activity is inhibited by UMP and activated by ornithine. CP is utilized by ornithine transcarbamylase (OTC) to form citrulline for arginine biosynthesis and by aspartate transcarbamylase (ATC) in the first step of pyrimidine synthesis. OTC is subject to genetic repression by arginine. ATC is repressed by pyrimidines and its activity is subject to feedback-inhibition by CTP and UTP and to activation by ATP. Monitoring these enzymatic activities in different balanced growth conditions showed that OTC activity is strongly repressed by the presence of arginine in the medium, but is increased by the presence of uracil; similarly, uracil increases the NAGS activity. This must reflect a physiological derepression, since this effect is not seen in an *argR*$^-$ strain. The hypothesis that uracil limits the synthesis of CP and its availability for arginine synthesis is supported by measurements of the cellular pools of UMP, ornithine, citrulline and arginine, using HPLC and LC-MS, and by the properties of a strain in which a very low constitutive synthesis of CP has been engineered.

U-P05 Comparative metabolomics of *Saccharomyces* yeasts

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Comparative analysis of gene sequence and gene content datasets from the genomes of closely-related yeast species reveals clear evidence of reticulate evolution (Holland et al 2004, Savva et al 2004). We are extending this work to examine network-like behaviour in “metabolic footprinting” datasets (Allen et al 2003, Lacey et al 2004). Networks derived from genomic and metabolomic data will be compared using combinatorial methods. The development of new dissimilarity measures will draw on previous work utilising similarity theory (Collins et al 2000, Dress et al 2002). This will reveal links between genotypic and metabolic variation and guide hypothesis-driven research into molecules and mechanisms responsible for the observed differences. Understanding such links will enable the results of comparative analyses to inform metabolic modelling.

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U-P06 Metabolic network analysis in six microbial species

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The structurally conserved and ubiquitous pathways of central carbon metabolism provide building blocks and cofactors for the biosynthesis of cellular macromolecules. The relative use of pathways and reactions, however, varies widely between species and conditions and some are not used at all. Based on stoichiometric models of the central carbon metabolism, we here identify the network topology of glucose metabolism and its *in vivo* operation by quantification of intracellular carbon fluxes from $^{1\,3}$C-tracer experiments. Specifically, we investigated *Agrobacterium tumefaciens*, two pseudomonads, *Sinorhizobium meliloti*, *Rhodobacter sphaeroides*, *Zymomonas mobilis* and *Paracoccus versutus*, which grow on glucose as the sole carbon source, represent fundamentally different metabolic life styles (aerobic, anaerobic, photoheterotrophs and chemoheterotrophs), and are phylogenetically distinct (firmicutes, gamma-proteobacteria and alpha-proteobacteria). When compared to the model bacteria *Escherichia coli* and *Bacillus subtilis*, metabolism in the investigated species differed significantly in several respects: i) the Entner-Doudoroff pathway was the almost exclusive catabolic route, ii) the pentose phosphate pathway exhibited exclusively biosynthetic functions, in many cases requiring also flux through the non-oxidative branch, iii) all aerobes exhibited fully respiratory metabolism without significant overflow metabolism, and iv) all aerobes used the pyruvate bypass of the malate dehydrogenase reaction to a significant extent. Exclusively, *Pseudomonas fluorescens* converted most glucose extracellularly to gluconate and 2-keto-gluconate. Overall, the results suggest that metabolic data from model species with extensive industrial and laboratory history are not representative for microbial metabolism, at least not quantitatively.

U-P07 Retrograde response to mitochondrial dysfunction is separable from Tor1/2 regulation of retrograde gene expression.

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To obtain insight into how retrograde (RTG) and target of rapamycin (TOR) signaling pathways integrate in response to specific stimuli in yeast cells, rapamycin sensitivity of the expression of the RTG target gene CIT2 and of two nitrogen catabolite repression (NCR) sensitive genes, GLN1 and DAL5, has been analyzed in rho^+ and rho^0 cells. Rapamycin treatment of rho^+ cells caused a 60-, 6- and 8-fold increase in the expression of CIT2, GLN1 and DAL5 expression, respectively. In rho^0 cells CIT2 expression is upregulated 13-fold compared with rho^+ cells. Rapamycin treatment of rho^0 cells increased CIT2 expression to the same level observed in rapamycin treated rho^+ cells. Mutations of LST8, a negative regulator of the RTG pathway and a component of TOR1 and TOR2 complexes, upregulated CIT2 and GLN1 but not DAL5 expression, which was induced by rapamycin treatment in wild type and lst8 mutant cells. Rapamycin-activated DAL5 expression was virtually abolished by deletion of either GLN3 or GAT1 GATA transcription factors. Subcellular localization analysis of Gln3p-GFP and Gat1p-GFP showed that Gat1p-GFP is completely excluded from the nucleus in lst8-5 mutant cells. The role of GATA factors in the RTG-dependent retrograde response was also studied. In both rho^+ and rho^0 wild type and gln3delta yeast cells, CIT2 expression was not affected by GLN3 deletion in rho^+ cells with or without TOR inhibition by rapamycin, whereas GLN3 deletion virtually abolished CIT2 upregulation due to retrograde response in rho^0 cells. TOR inhibition by rapamycin restored CIT2 expression at similar levels both in respiratory competent and deficient wild type and mutant strains. Reintegration of GLN3 by transformation of rho^0 gln3delta strain with pRS416-GLN3-GFP restored CIT2 expression. Thus the retrograde response in respiratory deficient cells follows a different pathway from that in TOR-regulated retrograde gene expression.

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U-P08 Unravelling new metabolic networks in LAB via the thioredoxin system

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Co-factors are produced by several micro-organisms and are often also essential components in human diet. The biosynthesis of these metabolites involves complicated metabolic pathways which represent a challenge for genetic engineering. Furthermore, identifying all the pathways where co-factors play a role in the cell metabolism requires an integrated approach. Hence, functional genomics-based approaches are being used to evaluate the effect of modulating production of a co-factor on the overall metabolism and functionality of lactic acid bacteria (LAB). The two LAB strains: *Lactococcus lactis* (2.3 Mb) and *Lactobacillus plantarum* (3.3 Mb) are used as the model bacteria, because of their available genome sequence, their scientific paradigm status, and the vast experience with these LAB strains in both practical application and biotechnological development.

The co-factor of choice is the redox-mediating thiol, thioredoxin. This oxido-reductase is involved in a broad spectrum of cellular processes such as DNA synthesis, protein folding, stress-response, and detoxification. In food this component can act as an anti-oxidant for stabilization of starter cultures and food proteins. Nevertheless, to be functional thioredoxin needs first to be reduced by thioredoxin reductase (TRXB) in a NADPH dependent reaction. Due to the essential role the reductase plays in the thioredoxin system, TRXB was further investigated.

Genetic modifications in the thioredoxin reductase *(trxB)* gene have lead to engineered strains in LAB will either increased levels of thiol or with no thiol at all. At the same time, enzyme assays have been optimized to quantify the thioredoxin and thioredoxin reductase activity. At the moment we have a construct that can over express the reductase activity approximately six times more than in the wild type. Furthermore, performed phenotypic characterization suggests that the over expression of thioredoxin reductase leads to better resistance towards diamide, a disulfide stress agent.

Currently, we are monitoring the mRNA response of these genetically modified LAB strains under different oxidative stress conditions. In the near future, we will study the impact of these modifications at the metabolome level. The data of these studies will be linked using metabolic-modelling tools such as Simpheny™. Undoubtedly, this functional genomics approach will contribute to our understanding of the crucial role of the thioredoxin system in Lb. plantarum.

U-P09 The regulatory circuitry of arabinases in *Bacillus subtilis*

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Hemicellulases are a diverse group of enzymes capable of hydrolyze hemicellulose, the second-most abundant renewable biomass polymer in nature. This fraction of plant cell walls comprises a complex mixture of xylans, arabinans, galactans and mannans. Enzymes responsible for degrading plant cell wall polysaccharides have many applications in different fields such as food technology, nutritional medical research, mobilization of plant biomass, plant biochemistry and organic synthesis. The saprophytic endospore-forming Gram-positive bacterium *Bacillus subtilis* produces two major enzymes, alpha-L-arabinofuranosidases (EC 3.2.1.55) and endo-alpha-1,5-arabinanases (EC 3.2.1.99), involved in degradation of the homoglycane arabinan, which are capable of releasing arabinosyl oligomers and arabinose from plant cell walls. In *B. subtilis*, arabinan is degraded by at least two extracellular hemicellulases, AbnA and YxiA. The resulting products, arabinose, arabinobiose, arabinotriose, and arabinooligosaccharides, are transported by different systems. Arabinose enters the cell mainly through the AraE permease, and the uptake of arabinose oligomers occurs most likely via AraNPQ, an ABC type transporter. These latter products might be further digested intracellularly by AbfA and Xsa. In previous studies our group showed that expression of the arabinases genes is regulated at the transcriptional level: (i) induction in the presence of arabinose and arabinan, (ii) repression by the transcription factor AraR, (iii) carbon catabolite repression by glucose, and (iv) temporal regulation (1). Here, we integrate our current knowledge concerning the regulatory network of arabinases with ongoing studies on the functional analysis and biochemical characterization of these enzymes.

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U-P10 Extending life by alternative respiration?

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Alternative oxidase transfers electrons directly from the ubiquinol pool in mitochondria to oxygen, allowing cell respiration in presence of complex III and IV inhibitors like antimycin A or cyanide. Electron transfer by alternative oxidase is not coupled with proton transfer across the mitochondrial membrane, thereby uncoupling the supply of small metabolic intermediates by the central metabolic pathway from energy production in the cell. Alternative oxidase is present in mitochondria of plants, many fungi and a few, mostly Crabtree-negative yeasts, but not in *Pichia angusta* (*Hansenula polymorpha*) and *Saccharomyces cerevisiae*. Alternative oxidase has multiple functions in different organisms. It is involved in stress answers, in programmed cell death, maintenance of the cellular redox balance, and also citric acid accumulation in *Aspergillus niger*.
We isolated the alternative oxidase gene from the methylotrophic yeast *Pichia pastoris* in order to study its effects on the cellular energy content, respiratory activity, its protective role against oxidative stress. Our results indicate the importance of an exact regulation of the alternative oxidase due to its impact on many cellular functions, especially cell viability.

U-P11 Systematic computational modelling reveals a key operating principle of TOR signalling in yeast

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The TOR (target of rapamycin) pathway is a highly conserved signalling route that couples nutrient availability to cell growth. Many of its components and their interactions are characterized, but, as a typical cellular regulatory circuit, the operating principles are not yet fully understood, in particular owing to conflicting hypotheses and experimental data. For TOR signaling in *Saccharomyces cerevisiae*, we report a quantitative analysis that systematically casts molecular hypotheses into a family of computational models and evaluates these against experimental observations. In contrast to the prevailing view of a *de novo* assembly of type 2A phosphatase complexes (PP2As), this approach proposed competitive binding of the phosphatases as a key signaling mechanism. Subsequent experimental analysis confirmed this prediction, thereby settling, for instance, apparent discrepancies between rapid PP2A activation an much slower substrate dephosphorylation. The comparative computational biology approach is broadly applicable, and can help unravelling the operation of incompletely characterized pathways.

U-P12 Dynamic on-line investigation of lactic acid bacteria

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Lactic acid bacteria (LAB) play a fundamental role in industrial food fermentation processes. In addition, their relatively simple sugar metabolism and high degree of auxotrophy present them as ideal model organisms for studying glycolysis and pyruvate derived pathways. Many studies have been conducted on LAB, but so far there are no unambiguous answers to the question of what controls the rate of glycolysis in this simple organism. A common feature of the investigations is that they maintain a constant pH during experiments of typically 6.5, thus counteracting the large acid production by the glucose consuming bacteria. Restraining pH allows for experimental simplifications, but also removes one of the key regulating factors of the system. We therefore find it crucial to be able to measure key metabolites continuously and quantitatively, during the large pH drop (from 6.7 to 4) normally observed during a standard milk fermentation (Oestlie, H ,*et al.*, 2003, Int J Food Microbiol, 87, 17). By correcting for pH changes instead of preventing them, we obtain data, which reflects the natural parameter space of the system. This allows us to investigate glycolysis under the physiological conditions, which govern the glycolysis of a LAB performing its primary function, namely acidification.
In our laboratory we focus on development and use of high throughput quantitative on-line methods for continuous monitoring of relevant biological compounds during sugar fermentation. These methods include Membrane Inlet Mass Spectrometry of gasses and volatiles, fluorescence spectroscopy of NADH and amperometric glucose electrodes. The methods have in common the ability to generate almost continuous data without any sample treatment or undesired perturbations to the system. These methods produces data well suited for kinetic modelling. They ensure a time scale that allows the results of minor perturbations to be studied in detail and exclude regulation on other levels than that of the enzymes. This way we hope to reveal the still elusive regulatory network governing glycolysis of this simple organism on an enzymatic level. Using the systems biology approach, the investigation and the resulting model can then hopefully be extended and merged with knowledge of proteomics, genomics etc. obtained under similar conditions in other laboratories.

U-P13 A dynamic model of cAMP signal transduction in yeast

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Yeast cells possess a number of signaling pathways to integrate information about nutrient supply with cellular growth and proliferation. The signaling route mediated by cyclic AMP (cAMP) and protein kinase A (PKA) regulates a large number of targets both at the posttranslational and transcriptional level in response to changes in carbon source availability. By affecting both metabolic processes and the cell cycle machinery, it also serves to coordinate cell growth and division.
Measurements were performed in synchronous cultures and in oscillating continuous cultures of *Saccharomyces cerevisiae* to analyze the cell-cycle dynamics of cAMP and energy metabolism. A modular single-cell model integrating cAMP signaling with descriptions of the cell cycle machinery and central carbon metabolism is currently under development. This single-cell model will permit to simulate cellular behavior resulting from the joint action of metabolic and signaling networks during the yeast cell cycle. The present contribution focuses on two models of cAMP signal transduction, which can be used as exchangeable submodules in the integrated model.
On the basis of an extensive literature survey two dynamic models of the cAMP signaling pathway were developed, both of which provide a comprehensive description of the current knowledge, but differ in the level of detail. They account for stimulation of adenylate cyclase via Ras and a GPCR system, cAMP destruction by phosphodiesterases, (in)activation of PKA, and for the negative feedback exerted by PKA on its own activity. Results of the above-mentioned experiments were employed in combination with literature data and stability constraints to estimate model parameters. As a starting point, protein levels determined in a genome-wide analysis [1] served as estimates of the initial values of model species. Simulation results of both the small-scale model (20 reactions) and the large-scale model (400 reactions) will be presented and compared to experimental findings.
The models provide a basis to address open questions regarding the underlying network structure and dynamic behavior of this signaling pathway. Plus, they can serve as a tool to identify suitable experimental conditions to efficiently discriminate between alternative hypotheses. Future work aims at incorporating spatial information and transcriptional regulation of key components of the cAMP pathway into the model.
[1] Ghaemmaghami, S., et al. (2003) Nature 425:737-741.

U-P14 Natural sweetening of food products: engineering *Lactococcus lactis* for glucose production

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Lactic acid bacteria (LAB) are used worldwide in the production of fermented dairy products. *Lactococcus lactis* is generally recognized as a model organism, characterized by the simplicity of its metabolism and the availability of a wide range of genetic tools as well as the availability of a complete genome sequence. These features make *L. lactis* a suitable object for metabolic engineering strategies aiming at the improvement of food quality and human health. Production of glucose from lactose to be used as a natural sweetening requires engineering the catabolism of glucose. A strain that ferments only the galactose moiety should be impaired in glucose transport and phosphorylation. Hence, a double mutant carrying specific deletions of the glucose PTS (*ptnABCD*) and glucokinase (*glk*) was made. However, this strain could still grow on glucose. Determination of enzymatic activities coupled to *in vivo* NMR studies of glucose metabolism suggested the presence of a second PTS system with a clear preference for alpha-glucose. A DNA microarray experiment revealed up-regulation of the genes coding for the cellobiose-PTS (*ptcBAC*). Indeed, deletion of *ptcBA* in the double mutant strain abolished growth on glucose. The lactose plasmid was introduced in triple knock-out mutant and in a control strain and lactose metabolism studied using *in vivo* NMR. In the control stain, both moeities were completely converted to lactate and a transient accumulation of galactose was observed. In contrast, in the mutant the glucose moiety is fully recovered as free glucose. Therefore, a *L. lactis* strain can be used to produce glucose as a natural sweetener in milk products was obtained. We showed that deletion of the genes coding for glucokinase, mannose-PTS and cellobiose-PTS is required and sufficient to completely block glucose metabolism and we identified the cellobiose-PTS as a glucose transporter in *L. lactis*.

U-P15 Adaptative response of the central metabolism in *Escherichia coli* to quantitative modulations of a single enzyme: glucose-6-phosphate dehydrogenase

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Microorganisms have an efficient capacity for adapting their metabolism in response to genetic or environmental changes, and understanding metabolic robustness has become an emergent issue. Part of the robustness originates from the network organization of metabolic systems, where the interplay between all available biochemical reactions provides alternative mechanisms for compensating the perturbations. Recently, ^{13}C-Metabolic Flux Analysis (^{13}C-MFA) has been applied to *E. coli* knock-out mutants lacking key enzymes to determine the phenotypic effects of structural changes in the metabolic network, providing further evidences for compensatory phenomena. The aim of our on-going work is to understand how the central metabolism in *E. coli* responds to quantitative alterations at a specific key-point of the metabolic network. The glucose-6-phosphate dehydrogenase (G6PdH), a key enzyme in the central metabolism for which the effects of deleting the gene (*zwf*) has been already described (*Zhao, J, et al.,(2004),Metab Eng., 6, 164*) was chosen as the target. To this aim we have generated a set of expression mutants, i.e. mutants having each a fixed level of expression of the *zwf* gene. Four different levels of expression, leading respectively to G6PdH activity 2;2,9;5,7 and 14 times higher than in the WT strain, have been obtained. For each mutant, transcriptomics analysis will be carried out and compared to both the *zwf-* and WT strains to detect changes in the network structure, and the distribution of fluxes will be measured using ^{13}C-MFA. The flux maps obtained for the various strains will be compared to evaluate the quantitative response of the central metabolic network to imposed and increased G6PdH activity. Metabolic control analysis will be applied to provide insights onto the sensitivity of the measurable metabolic fluxes to G6PdH activity. Combination of transcriptomics and fluxomics approaches will provide information on the nature and extent of the compensatory mechanisms. Because the activity of a single enzyme is tuned at different levels in knock-out and expression mutants, this investigation provides a situation that mimicks gene-level regulation of metabolism.

U-P16 Stress induced by weak organic acids in *Saccharomyces cerevisiae*

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Sorbic acid, a weak organic, is the most widespread food preservative used in the industry. Yeast and other fungi are, to a certain extent, able adapt to this acid and resume growth in the presence of the highest concentrations allowed in foods. This can result in substantial economic losses. Quite a lot is know how yeast responds to sorbic acid stress at the genetic level from transcript data and using the yeast knockout collection, but we still do not understand why the cell arrests growth, and why, after adaptation, it can resume growth. Therefore, to understand the mechanisms of growth limitation and adaptation we study yeast at the level of gene expression, protein composition, but mainly at the metabolic level. By calculating energy generating capacity, we try to map the cost and benefit of the various aspects of the stress response. In practice this means we determine metabolic fluxes, ATP/ADP ratios and ultimately try to construct a mathematical model of the response to the stress. We think that the combination of physiological, protein and gene-expression data will provided us with a more complete understanding of the mechanisms underlying the stress and adaptation.

U-P17 Adaptation of yeast glycolysis to temperature changes

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Organisms are able to respond to their environment to maintain homeostasis. A well-studied environmental parameter is temperature, which exerts a complex combination of effects on the cell. We have studied the relation of growth rate and temperature of *S. cerevisiae* under well-defined conditions and focused on the effect on glycolysis. We selected a temperature from the supra-optimal side of the temperature growth rate graph and shifted a culture from the reference temperature to a higher temperature (38ºC) to examine quantitatively the effects on glycolytic flux during adaptation. We observed a twofold increase in glycolytic flux. Now we want to find out how this flux increment is regulated; is it regulated metabolically, through temperature effect on the glycolytic enzyme reaction rates, through variations in metabolite concentrations, or is it regulated hierarchically, at the level of mRNA levels, protein levels, through the expression of alternative iso-enzymes. We will use regulation analysis to determine this for all enzymes in the glycolytic pathway, and will then fully study interesting cases on all levels, from gene to function...and back.

U-P18 Progressive adaptation of *Lactococcus lactis* to stress

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The bacteria *Lactococcus lactis*, recently sequenced and recognized as the model organism for the study of lactic acid bacteria, is encountered in various environments (industrial dairy media, natural vegetal ecosystems, human digestive tract) in which it is submitted to multiple growth-limited stresses (nutrient starvation, thermal, oxidative, acidic or osmotic stresses). Surprisingly, mechanisms of adaptation against these adverse environments are poorly characterized and fundamental knowledge is still missing. As no alternative sigma factor involved in stress adaptation has been identified in its genome, regulation of response towards environment may differ in *L. lactis* from what is observed in the Gram$^+$ model *Bacillus subtilis*.
To study the *L. lactis* response to adverse growth conditions, a vertical approach ranging from transcription to phenotype is followed. During controlled cultures (pH, temperature, medium composition), a stress is progressively imposed to observe and analyse the dynamic adaptation of *L. lactis*. Throughout the culture, whole-genome messengers expression is measured and integrated in a global physiologic analysis based on particular metabolic indicators (metabolic rates, enzyme concentrations, amino acid consumptions). In order to evaluate the mRNA turnover impact on the overall regulation, mRNA half-lives are also measured and analysed with the transcriptome data (*Even, S., et al., (2002), Mol Microbiol, 45, 1143*). This complete approach allows to quantify the various determinants of the response regulation and identify the major bottlenecks (*Even, S., et al., (2003), Microbiology, 149, 1935*).
One of the major stress investigated in *L. lactis* was glucose starvation (*Redon, E., et al., (2004), J Bacteriol, submitted*). After a rapid exponential phase associated to nutrient excess and a short deceleration phase due to decreasing glucose concentration, succeeded a stationary phase characterized by glucose starvation. The transcriptional response triggered early in the fermentation, notably before glucose exhaustion, and varied slightly once starvation was established. 704 genes (30% of genome) were shown to be involved in carbon starvation adaptation. Their functional analysis allowed to identify different types of response, providing a deep understanding of the mechanisms involved in stress adaptation.

U-P19 Evolutionary conservation and divergence of fungal promoter sequences

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The recently sequenced and fully annotated genome of the filamentous fungus Ashbya gossypii revealed striking similarity to the baker's yeast *Saccharomyces cerevisiae*. 90% of A. gossypii genes share homology and also a substantial degree of synteny (gene order conservation) with S. cerevisiae. Although both organisms originate from the same ancestor (carrying about 5000 protein coding genes), the evaluation of synteny was complicated by the fact that their evolutionary paths included not only about 300 translocations and inversions but also a whole genome duplication in the *S. cerevisiae* lineage followed by loss of 4000 genes. As a consequence the alignment of the A. gossypii genes with homologous *S. cerevisiae* genes results in many synteny clusters in which one A. gossypii chromosomal region aligns with two chromosomal regions of S. cerevisiae. The clusters themselves are made of gene regions displaying relaxed (incomplete) and stringent (complete) synteny. The latter is found in many small regions of up to eleven genes which, very importantly, are not interrupted by end points of rearrangements. Thus, these regions are particularly suitable for investigations of evolutionary conservation and divergence of syntenic sequences which started diverging over 100 million years ago. In the past, most studies of syntenic regions looked into conservation and divergence of open reading frames (ORFs) and the proteins they encode. We have started an investigation of evolutionary selection regarding size and sequence of inter-ORF regions. A detailed discussion of the subject will be presented taking into account DNA-binding sites of transcription factors, transcription start and terminator sites and inter-ORF lengths discerning between bidirectional or unidirectional promoters and pure terminator-bearing inter-ORFs.

U-P20 A Systems Biology approach for the optimization of recombinant protein production in *E. coli*

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Escherichia coli has been the organism of choice for the production of many recombinant proteins with high therapeutic value. However, while the research on molecular biology has allowed the development of very strong promoters, there are still several phenomena associated with this process that have hampered the full use of that promoter strength, namely the aerobic acetate production associated with high specific growth rates. The presence of acetate is known to reduce both biomass yield on the chosen carbon source and protein productivity while totally inhibiting growth when present at high concentrations due to its toxic effect. While there have been several studies covering the recombinant protein production process with the bacterium Escherichia coli, including genome-scale analysis of the transcriptome, proteome, fluxome or metabolome, there has been a lack of an integrative approach that is able to combine genomic and physiological information about those processes with high-throughput analysis.
Also, the existence of genome-scale models that cover both stoichiometry and regulation of some pathways has not been taken into account in genome-scale data analysis and for the consequent formulation of hypothesis and development of new strategies for improving the performance of the process.
In our group, a high-cell density fed-batch process for recombinant protein production in *E. coli* is being studied, giving particular relevance to acetate production. A systematic approach is being used, by first compiling the existing knowledge about this phenomenon, extending existing genome-scale models to accommodate that knowledge, derive hypothesis in silico that are then tested by using genome-scale analysis of the omes. A reliable fermentation process was developed to be able to reproducibly study this phenomenon in different strains in order to reduce external variances to a minimum.

U-P21 Some properties and partial purification of ICandida Guilliermondii NP-4/I and IParamcium Multimcronucleatum/I glutaminase

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Have been investigated the activity of enzyme glutaminase in yeasts *Candida guilliermondii* and in aerob parameciums *Paramecium multimicronucleatum*.
The investigations show that the general activity of glutaminase is concentrated in the mitochondrias (about 85%), this mitochondrial glutaminase is phosphate depended. Also there is a low level of glutaminase activity in the nucleus (about 15%), and in the mycrosomal fraction (1%), this glutaminases were phosphate dependent.
The investigations show two isoensymes of glutaminase in yeasts *Candida guilliermondii*. The phosphate independed isoglutaminase was located in the nucleic fraction of cells (about 30%). Mithochondrial (70%) and mycrosomal (2%) glutaminase of yeasts *Candida guilliermondii* were phosphate dependent. pH optimum for yeasts glutaminase was 8,5.
Have been investigated the effects of different activators and inhibitors on the mitochondrial glutaminase of yeasts *Candida guilliermondii* and parameciums *Paramecium multimicronucleatum*.
The mithochondrial glutaminase of *Paramecium multimicronucleatum* have been partrial purified by the method of the ionexchange chromatography (using gradiative elution by different concentrations of NaCl. Have been shown, that more than 80% of total glutaminase activity of *Paramecium multimicronucleatum* are located in the mithochondrias of the cells. Two isoensymes of the glutaminase have been revealed in the mithochondrias of *Paramecium multimicronucleatum*. The total activity of glutaminase I have been 45.26 ± 1.51 mM ammonium (specific activity - 22.86 ± 1.12 mM ammonium/ prot.). The total activity of glutaminmase II have been 13.61 ± 1.02 mM ammonium (specific activity - 17.01 ± 1.08 mM ammonium/ prot.). The activity of the glutaminase I makes about 75% ot total glutaminase activity of mithochondria. The degree of enzyme purification has been 26 (72 %). K_m for glutamine of the glutaminase I has been 4.3 X 10^{-4} M, for glutaminase II - 5.5 X 10^{-4} M. K_i for glutamic acid of glutaminase I was 1.5 X 10^{-5} M, for glutaminase II - 2.5 X 10^{-6} M. Have been shown that glutaminase I is phosphate dependent, while glutaminase II is phosphate independent, it is positively regulated by citrate. ATF, tiroxine, dexametazone, adrenaline and bicarbonate have show the positive effect on activation of both mitochondrial isoglutaminases.

U-P22 Unusual group II introns in bacteria of the Bacillus cereus group

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A combination of sequence and structure analysis and RT-PCR experiments was used to characterize the group II introns in the complete genomes of two strains of the pathogen *B. cereus*. While *B. cereus* ATCC 14579 harbors a single intron element in the chromosome, *B. cereus* ATCC 10987 contains three introns in the chromosome and four in its 208-kb pBc10987 plasmid. The most striking finding is the presence in *B. cereus* ATCC 10987 of an intron (B.ce.I2) located on the reverse strand of a gene encoding a putative cell surface protein which appears to be correlated to strains of clinical origin. Because of the opposite orientation of B.ce.I2 the gene is disrupted. Even more striking is that B.ce.I2 splices out of an RNA transcript corresponding to the opposite DNA strand, a situation never observed before. All other intragenic introns studied here are inserted in the same orientation as their host genes and splice out of the mRNA in vivo setting the flanking exons in-frame. Noticeably, B.ce.I3 in B. cereus ATCC 10987 represents the first example of a group II intron entirely included within a conserved replication gene, namely the alpha subunit of DNA polymerase III. Another striking finding is that the observed 3 prime splice site of B.ce.I4 occurs 56 bp after the predicted end of the intron. This apparently unusual splicing mechanism may be related to structural irregularities in the 3 prime terminus. Finally, we also show that the intergenic introns of *B. cereus* ATCC 10987 are transcribed with their upstream genes and do splice *in vivo*.

U-P23 The effect of oxygen tension on yeast glycolysis

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The catabolism of *S. cerevisiae* ranges from a highly efficient, respiratory mode with carbon dioxide as sole end product, to a fully fermentative mode that results in high specific rates of sugar conversion to carbon dioxide and ethanol. Fully respiratory growth is only found when oxygen is saturating and glucose availability is limited, while fully fermentative growth is found under strictly anaerobic conditions. Intermediate situations, characterized by a mixed respiro-fermentative growth, are found either when the oxygen availability is limited, or when well-aerated cultures are subjected to glucose excess. The transition between respiratory and fermentative catabolism is known to involve various levels of the cellular regulation hierarchy. However, the relative importance of the various regulation mechanisms is not known let alone quantitated.
In this project the regulation of these transitions is investigated in a time-resolved manner at the metabolic, the enzyme activity, the proteomic and the transcriptomic levels. To induce these transitions, *S.cerevisiae* CEN.PK 113-7d is cultivated in steady-state glucose-limited chemostat cultures under various oxygen regimes (fully aerobic, hypoxic, microaerobic and anaerobic). After quantification of the oxygen availability for a given experimental set-up, the experiments will focus on a quantitative description of the cell in steady state (a snapshot) with respect to gene expression, enzyme activity, metabolite concentrations and specific metabolic activity. Quantification of these regimes necessitates calibration of the experimental set-up. Subsequently, the changes in cellular make-up and activity will be monitored (a movie) when it adapts from one steady state to the other. This approach will allow for the discrimination between cause and effect during adaptation. The use of well-defined steady state conditions will yield a data set that is sufficiently quantitative for modeling of the response.

U-P24 Vertical genomics in baker's yeast: adaptation of respiring cells to anaerobic sugar-excess conditions.

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The primary role of bakers' yeast (*Saccharomyces cerevisiae*) in the leavening of bread dough is the production of carbon dioxide via the alcoholic fermentation of sugars. Within this process an important parameter is the fermentative capacity, defined as the specific rate of carbon dioxide (and ethanol) production immediately upon introduction of yeast into dough (3). However, alcoholic fermentation is highly undesirable during the industrial production of bakers' yeast, as it reduces the biomass yield on the carbohydrate feedstock. Industrial bakers' yeast production is therefore, performed in aerobic, sugar-limited fed-batch cultures. Hence, conditions during production of bakers' yeast differ drastically from the dough environment, which is anaerobic and initially contains an excess of sugars. This project undertakes a Systems Biology approach to ultimately understand and control the regulatory mechanisms (from gene to flux) that govern the induction of fermentative capacity. This project is part of a larger IOP-Genomics program "Vertical Genomics", which is a collaboration between six research groups from different universities (TUDelft, UvA and VUA).
The dynamic control of both fermentative capacity and fermentative activity will be simulated and analysed in detail. Aerobic glucose-limited chemostat cultures will be used to simulate a baker's yeast production process. Chemostat cultures are preferred over fed-batches, as the culture conditions can be defined and tightly controlled. The dynamic dough environment is simulated by shifting the chemostat to anaerobic batch conditions, followed by addition of a glucose pulse. To understand at which cellular 'level(s)' the fermentative capacity, and specifically glycolysis, is regulated (*i.e*. transcriptional or post-transcriptional control) the experimental approach includes analysis at various regulatory levels, *i.e*. transcript level (micro-array/ qPCR), protein levels (protein chips/ MS), enzyme activity assays and *in vivo* carbon-fluxes with stoichiometric modelling. Diverse datasets will be aligned, compared and integrated, and corellations between regulatory levels will be mapped.
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U-P25 LacplantCyc: in silico reconstruction of the metabolic pathways of *Lactobacillus plantarum*.

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Lactobacillus plantarum is a versatile and flexible lactic acid bacterium (LAB) that is important in industrial food fermentation processes. It is also one of the LAB species that are important as probiotics in health-promoting food products. We have sequenced and annotated the genome of *L. plantarum* WCFS1 (PNAS USA 2003; 100:1990-1995), which is one of the largest Lactobacillus genomes. *L. plantarum* serves as a model organism for genome annotations and comparisons with other LAB (lactobacilli, lactococci, streptococci). This work describes a reference database of the metabolic network, based on the Lactobacillus genome annotation.
LacplantCyc is a pathway / genome database (PGDB) describing the entire genome as well as its biochemical pathways, reactions, and enzymes. This database was automatically generated from the annotated *L. plantarum* WCFS1 genome and the MetaCyc database (Nucleic Acids Research 2004; 32:D438-42) using the PathoLogic software from Pathway Tools (Bioinformatics 2002; 18:S225-32). LacplantCyc was subsequently curated manually: transporters were added, new pathways were created and others updated.
LacplantCyc adds an extended dimension to the genome of *L. plantarum*, providing researchers with a helpful tool for the analysis of the genomic, proteomic, and metabolic information of the organism. Visualization of the data sets in different levels of detail is extremely important to help interpreting these data from a biological viewpoint. Once the connections between genes, proteins and reactions in a metabolic map have been defined, high-throughput transcriptome data can be projected on metabolic maps. An additional aim during this process is to formulate which information is useful for improved pathway reconstruction. This should speed up the reconstruction of the metabolic networks of other LAB of which the complete genome has been sequenced. In this way LacplantCyc is envisioned to become the reference Gram-positive PGDB for LAB. It is the first well-curated metabolic pathway database for Gram-positive bacteria in general, and lactic acid bacteria in particular. *LacplantCyc is available at http://www.lacplantcyc.nl.*
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U-P26 HIGH-THROUGHPUT screening of *Saccharomyces cerevisae* knockout library: method development and stoichiometric profiling

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The present work aims at the method development for high-throughput screening and stoichiometric profiling of *Saccharomyces cerevisiae* mutants. For selected gene deletion mutants, we studied kinetic and stoichiometric profiles (growth rate, respiration, yields of biomass and ethanol) at miniaturized scale by cultivation in 96-well microtiter plates. Cultivation included on-line sensing of dissolved oxygen by immobilized fluorescence sensors. Loss of ethanol and water due to evaporation during cultivation was corrected by using a dynamic model. Cultivation at this micro-scale displayed growth profile, substrate consumption and product formation patterns highly similar to conventional shake flask cultivation. Furthermore, we have investigated the effect of shaking rate on oxygen limitation. Mutants, cultivated on glucose, fructose and galactose showed substrate specific differences in specific growth rates and yields. Comparative phenotypic profiles in different environments allow a detailed classification of mutants. First experiments involved tracer studies with 1-13C labeled glucose, fructose and galactose. Labeling patterns of mutants were different on the different carbon sources, giving a first impression of differences in the underlying fluxes. We propose the utilization of the developed methodology for large-scale quantitative screening of yeast deletion mutants.

U-P27 A Systems Biology Strategy For Understanding The Genome-wide Control Of Growth Rate And Metabolic Flux In Yeast

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Baker's yeast, *Saccharomyces cerevisiae*, was the first eukaryotic organism to have its genome sequenced. This makes it a central 'model' organism in modern biology, and it is important to know what controls the rate at which it can grow. Moreover, yeast is an industrially significant organism from many points of view. Some industries (e.g. baker's yeast production) wish to maximise growth rates, others (e.g the antifungal industry) to minimise them. We are engaged in the development of both top-down and bottom-up genome-wide models for the control of the maximum specific growth rate in *S. cerevisiae*. Genes with high flux-control coefficients will be identified via haploinsufficiency measurements in turbidostats, and will form the components of a coarse-grained model in the top-down approach. Intra- and extra-cellular metabolome transcriptome and proteome measurements in selected genetically defined strains will be used, iteratively, to validate models. Flux-balance modelling will also be used to define specific modulations likely to be most discriminatory between competing models. The result will be the first example in which the controls on growth rate and metabolic fluxes are established on a genome-wide scale.

Power posters

U-PoP1 Modeling and analyses of *Mycobacterium tuberculosis* metabolism

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Tuberculosis (TB) disease remains a serious problem that threatens human health around the world. TB prevention and treatment has been hindered by multi-drugs resistant TB (MDR-TB) mainly emerged from the lengthy drug treatment, and the lack of discipline of TB patients. This lures us to seek for new drug targets for further drug design and development. In this study, we reconstructed the species-specific metabolic pathways network of *Mycobacterium tuberculosis* H32Rv (MTB) from its complete genome sequence information and from various literatures. We employed 2 different approaches to analyze and model the network to identify potential anti-TB enzyme targets. The first approach was based on the analysis of network topology using elementary flux mode analysis. METATOOL software was used to determine all possible elementary routes for the synthesis of mycolic acid, an important component of cell envelope of MTB. Enzymes that were present in every elementary route were considered the key drug targets for TB because the lack of these enzymes would not lead to synthesis of mycolic acid. It was found that the enzymes, inhA, accD6, kasA, kasB, cmaA2, pcaA, fabD-Pacp, accD4, accD5, accD3, desA1, desA2, desA3, dcb, mmaA1, mmaA2, cmaA1 and acrA1 are potential drug targets for TB disease. The second approach used the metabolic network information to build a genome-scale metabolic model of MTB using flux balance analysis. This MTB model integrated 473 genes and 419 metabolites with 601 biochemical reactions. The model was used to perform *in silico* single gene knockout. From a total of 21 genes, which were previously identified as drug targets, 18 cases led to the fatality of the *in silico* MTB, indicating an 85% model accuracy. From the *in silico* gene knockout experiments of all remaining genes, we identified a list of 91 essential genes whose protein products are promising targets for TB drugs. We found that most targets identified by both approaches are in good agreement. Among those, a few will be chosen for further experimental validation and future steps in the development of new drugs for multi-drug-resistant (MDR) strains that have caused millions of deaths worldwide.

U-PoP2 SOME CHANGES IN THE COMPOSITION OF NUCLEAR COMPONENTS DURING CEREAL SEEDS GERMINATION.

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Most eukaryotic genomes are packaged into two constitutive regions of chromatin: euchromatin and heterochromatin. Heterochromatin represents significant fraction of most eukariotic genome and its function remain unknown. Nuclear pore complexes (NPC), including in nuclear membrane also appear to control the spatial orientation and transcriptional activity of chromatin. In yeast, NPC influence telomeric silencing via fiber-forming proteins emanating from their nucleoplasmic face. Recent studies have also shown that tethering a gene to the NPC can prevent its repression by creating a boundary between the gene and surrounding silenced heterochromatin
Our investigations shown changes in some physico-chemical characteristics of cereal seeds DNA, chromatin and nuclear membrane during genome activation. We have suggested that the high methylated region of repeated DNA commonly lies in heterochromatin region adjacent to the nuclear envelope (NE).
Recent studies have support that the NPC through association with the underlying chromatin regulates gene expression. Particularly have been obtained the changes in the DNA, RNA, protein and phospholipid content of the NE, soluble nuclear fraction and chromatin during germination of cereal seeds embryos and under influence of exogenous gibberellin A3. The characteristic trait for growing seed nucleus is a rising of protein and phosphatidic acid in nuclear membrane content to the third day of growing. The chromatin separated on the euchromatin and heterochromatin constitutive regions, and subsequently obtained parameters of thermal denaturation and the level of DNA methylation. Have been revealing changes in all abovementioned parameters during seed germination and under influence of gibberellic acid A3.
The goal of this work is to show correlation between genome activation and the change in content of the nuclear membrane during genome expression. Nevertheless much about the relationship between chromatin organization and the NE remains to be discovered.

U-PoP3 Differentiation in a genetic network with duplicate repressors: simulating evolutionary pathways based on Lac mutational data

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To add and alter functions, gene regulatory networks must be able to make new interactions and break old ones. A much-debated concept for such network “re-wiring” is the differentiation of duplicate regulatory components. However, we still lack a quantitative insight in the underlying evolutionary pathways at a mutation-by-mutation level. Here, extensive mutational data of the Lac system is integrated into a computer simulation scheme to systematically study such pathways. In a network with duplicate repressors and their binding sites, recognition is initially indiscriminate and must evolve towards independent binding. We find the paths to be surprisingly short and without detours, with the fitness increasing rapidly and almost immediately. In an alternative scenario, where a new operator must emerge from a random sequence, chances of success are much reduced because prolonged neutral drift is required. The fitness landscape as revealed by the rapid pathways appears diverse, with steep cliffs but also smooth regions. These landscape features, together with a cooperation between repressor copies appear to be key for the system’s evolvability. The presented approach can be more broadly applied to render network evolution simulations more experimentally relevant, and provides key insight for new experiments where natural as well as artificial networks are shaped by directed evolution.

Multicellular/mammalian

Posters

M-S01 Inferring feedback mechanisms in cellular transformation due to oncogenic RAS

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Intracellular signaling cascades display distinct activation profilesin response to various stimuli. Such activation patterns are strongly influenced and shaped by feedback loops. Different feedback loops can act in a cell context- and stimulus-dependent manner and produce a variety of temporal activation profiles, including oscillations and hysteresis. The MEK-ERK cascade plays an important role in cell-cycle regulation, differentiation and in cell transformation caused by oncogenic RAS. This cascade is regulated by several positive and negative feedback loops and is essential for signal transmission due to many different stimuli. While post-translational feedback loops have been subject to extensive mathematical modeling, feedbacks that are mediated by transcriptional control are still poorlyunderstood.

Using a combination of time-course experiments, mathematical modeling and bioinformatic analysis we investigate the effect of transcriptional feedback regulation in cellular transformation following induction of oncogenic RAS. In fibroblasts harboring an inducible RAS oncogene, we monitor the phosphorylation of ERK1,2 by Western Blot analysis. In addition, we analyze the expression profiles of RAS target genes with microarrays in a time-resolved manner. The phosphorylation of ERK shows a biphasic response upon constant induction and an oscillatory response after brief induction of RAS. We find that several dual specific phosphatases are expressed with similar kinetics. A bioinformatic analysis unveils two ERK-dependent transcription factors that control this battery of phosphatases. Together with the transcription factors, these phosphatases constitute a negative feedback for ERK-activity. Mathematical modeling and experimental interference shows that we can explain the biphasic and oscillatory dynamics as a result of phosphatase activation.

M-S02 Regulation of MAPK signalling determining cell fate in PC-12 cells - a step beyond biochemistry

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Mitogen activated protein kinase (MAPK) cascades participate in a wide array of cellular transduction programmes including cell growth and division, movement, differentiation and cell death. A paradigm system to study how the activity of these cascades produces different cell responses is the PC-12 cells system. In these cells the classical ERK pathway is activated by both EGF and NGF, giving rise to cellular opposite fates – division and differentiation, respectively. We believe different biochemical topology may be the key determining these specific responses. We are therefore interested in measuring reaction states of main components of this pathway, to analyze how the kinases are spatially organized and biochemically connected. We are using polychromatic fluorescence activating cell sorting (FACS) with phospho-labelled antibodies, which detect the active state of network components. By applying systematic perturbations of activities and subsequent read out on multiple reaction states at steady-state we are able to retrieve information on the network topology. Single cell measurements are being performed and RNAi and pharmacological inhibitors used for the perturbations. Moreover, response coefficients for each kinase, before and after perturbations will be calculated and first order connectivity maps built.
In addition, by using fluorescence resonance energy transfer (FRET) imaging with multiple optical sensors, reaction states of kinases and their spatial information are being determined simultaneously in one cell. Fusion proteins of GFP mutants and pathway kinases allow the detection of protein-protein interactions and molar ratios of phospho-proteins can be detected, by using phospho-antibodies against phospho-residues on active kinases.

M-S03 Mathematical modeling of neuronal response to neuropeptides: Angiotensin II signaling via G-protein coupled receptor

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In neurons G-protein coupled receptors (GPCRs) are involved in the alteration of neuronal activity (neuromodulation) via cascades of interacting proteins. The complex dynamic behavior of these networks, e.g. the integration of different signals, cannot be understood by intuition alone. Mathematical modeling provides an appropriate tool to decipher this complexity. Angiotensin II and AT1 receptor dependent signaling was investigated as an examples that use GPCR signaling pathways (Gq). AT1 signals via a wide variety of intracellular signaling molecules, involving (1) G-protein mediated stimulation of phospholipase C (PLC), with subsequent Ca2+ mobilisation; (2) Jak/STAT pathway; (3) transactivation of tyrosine kinase pathways. Relevant signaling outputs are modified gene expression patterns and modified neuronal activity via changes in membrane ionic currents and firing rate.
New data that was collected recently [Fernandez et al., Hypertension Jan.2003:56-63] showed that Angiotensin II can elicit stimulating and suppressive effects in the same neurons in dependency of the basal Ca2+ level. We have built a detailed mechanistic model of Angiotensin II signaling that captures both the stimulating and suppressive effects. This ODE model includes the AT1 mediated activation of PLC and PKC, and IP3 and channel mediated variation of the cytosolic Ca2+ level after Angiotensin II stimulation (adapted from [Mishra and Bhalla, Biophys. J., 83:1298-1316, 2002]). Based on in silico simulations of this model, we hypothesize that the observed biological variability is based on cell-to-cell variation in the dynamics of the Na-Ca exchanger.
Furthermore, a Hodgkin-Huxley model approach is used to investigate the function of cell signaling in altering the firing behavior of NTS neurons in response to various baroreceptor stimuli. Angiotensin II was found to activate neuronal firing in low firing NTS neurons.
In summary, detailed mathematical is a valuable tool to understand and investigate neuronal response to neuropeptides and furthermore to link signal transduction to the electrophysiological behavior of neurons.

M-P01 Control of the ATP/ADP ratio in pancreatic beta cells

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Pancreatic beta cells respond to rising blood glucose concentrations by increasing their oxidative metabolism, which leads to an increased ATP/ADP ratio, closure of K_{ATP} channels, depolarisation of the plasma membrane potential, influx of calcium and the eventual secretion of insulin (Rutter, G, (2001), Mol. Aspects Med., 22, 247). Such a signalling mechanism implies that the ATP/ADP ratio in beta cells is flexible, which is in contrast to other cell types (e.g. muscle) that maintain a stable ATP/ADP poise whilst respiring at widely varying rates. To determine whether this difference in flexibility is accounted for by mitochondrial particularities, we are currently performing a top-down metabolic control analysis to assess quantitatively how the ATP/ADP ratio is controlled in mitochondria isolated from rat skeletal muscle and cultured beta cells. We have defined the experimental system to contain two explicit intermediates, the protonmotive force and the external ATP/ADP poise, through which electron transfer, phosphorylation, proton leak, and ATP-consuming (i.e. added hexokinase) reactions interact. The elasticities of these processes to both intermediates are determined by the multiple-modulation method, and control values are calculated from the elasticities (Cornish-Bowden, A, et al., (1994), Biochem. J., 298, 367).
Preliminary measurements of oxygen-uptake activity and membrane potential suggest several differences between the mitochondrial energetics of muscle and beta cells. For example, the basal proton leak in muscle mitochondria is reduced, at every membrane potential measured, by ATP as well as ADP. Neither nucleotide affects proton leak in the presence of carboxyatractyloside, suggesting the effects are mediated by the adenine nucleotide translocator. In beta cell mitochondria, however, proton leak is not affected by ATP or ADP. Furthermore, the activity of the respiratory chain in beta cell mitochondria is lowered significantly when the organelles are depleted of adenine nucleotides, which is not the case in muscle. Also of potential relevance is the observation that the mitochondrial sample obtained from beta cells, unlike that from muscle, exhibits hexokinase activity. Data will be presented to reveal the extent to which the observed differences between muscle and beta cell mitochondria are reflected by the way the ATP/ADP ratio is controlled in these systems.
This work is supported by the Medical Research Council.

M-P02 Regulation of the INF-Gamma/JAK/Stat1 signal transduction pathway

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The STATs (Signal Transducers and Activators of Transcription) constitute a family of seven transcription factors regulating a multitude of cellular functions like immune reactions, growth, proliferation, differentiation and apoptosis. In response to stimulation by extracellular signaling proteins, such as cytokines and growth factors, the STATs are transiently activated by phosphorylation through receptor-bound Janus kinases (JAKs) and accumulate rapidly in the nucleus, where they switch on expression of their target genes. Here we present a detailed mathematical model of IFN-Gamma/JAK/Stat1 signaling, which is able to reproduce the behavior of this pathway in a quantitative manner for wildtype Stat1 and three Stat1 mutant proteins under various exeprimental conditions.
Parameters of the model were adjusted to independent experiments of wildtype Stat1 and mutant proteins. The import mutant shows no nuclear import of phosphorylated Stat1 and is dephosphorylated significantly slower. A second mutant is rapidly exported to the cytoplasm both in the phosphorylated and unphosphorylated form. Using data from experiments with these proteins, parameters for dephosphorylation and transport rates could be fitted. With this parameter set we were able to reproduce quantitatively the phosphorylation kinetics and subcellular distribution timecourses under stimulation with IFN-Gamma and for a number of pharmacological protocols using transport and kinase inhibitors.
Using the model we analyze the properties of Stat1 signal transduction by computing the control of the individual pathway steps on the amplitude and duration of the nuclear phosphorylation signal for different stimulation patterns. The analysis reveals that for weak stimulation conditions the amplitude is primarly governed by receptor-driven processes, DNA binding and the nuclear phosphatase. Under strong stimulation the phosphatase retains its high negative effect. The most significant control is now exerted by nuclear import and export of inactive Stat1. The residence time of phopshorylated Stat1 in the nucleus is negatively influenced by the nuclear phosphatase, too. DNA binding and receptor half-life exert significant positive control on the signal duration. We also found an intrinsic response time of the system, during which variations of ligand occupancy of the receptor are not transduced to the nuclear compartment.

M-P03 Comprehensive analysis of the cancer Tyrosine Kinome & Phosphatome

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Protein tyrosine kinases and phosphatases play crucial roles in the regulation of cellular processes like proliferation, differentiation, motility and survival. Receptor type tyrosine kinases (RTKs) function as signal transmitters across the plasma membrane by integrating a multitude of extracellular stimuli. Protein tyrosine phosphatases (PTPs) are the negative regulators of these processes through the removal of phosphate groups from tyrosine residues in RTKs and downstream signal proteins. Disturbances in this tightly controlled system have been shown to be causally connected to a variety of pathophysiological phenomena such as cancer. The largest group of genes with oncogenic potential belong to the PTK family and PTPs have by definition a tumor suppressor function which however, is largely unproven.
Cancer represents a genetic disease that begins with a series of damages in the genome of one cell in the form of DNA modifications such as point mutations, rearrangements or sequence amplification affecting regulatory genes such as PTKs. For example a point mutation in the transmembrane domain encoding region has been found to cause constitutive activation of the neu RTK and to induce cancer in rats [1]. Moreover, mutations in the FGFR3 gene have been associated with bladder and cervix cancer in humans [2]. Breast cancer patients with high expression of the FGFR4 gene and a single nucleotide change showed accelerated disease progression and reduced overall survival [3].
The aim of our study is the analysis of all PTKs, PTKLs as well as PTPs and DUSPs by direct sequencing of cDNA from cancer cell lines and primary tumors in order to identify so far unknown oncogenic or tumor suppressing mutations. Therefore we designed gene–specific primers to generate overlapping PCR fragments of about 900bp of each PTK/PTP. RNA was isolated from cancer cell lines and tissues, reverse transcribed and the cDNA is used as template for PCR to generate fragments for sequencing.
Identified genetic alterations will be verified in clinical tumor samples, correlated clinico-pathological parameters and functionally characterized in appropriate cell systems for their ability to influence transformation-characteristic cell properties like contact inhibition, colony formation in semi solid agar, migration, invasion and tumorigenesis in mice

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[3] *Bange et al., (2002) Cancer Res, 62, 840.*

M-P04 Sensitivity analysis with respect to initial values of the TNFalpha mediated NF-kappaB signalling pathway

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Objective The objective of this work is to investigate the behaviour of the TNFalpha mediated NF-kappaB signalling pathway. Starting from a mathematical model of the pathway consisting of ordinary differential equations, we studied derivatives of the solution of the model with respect to its initial values.
Methods The mathematical pathway model presented by Cho etal in [1] was taken as basis to build up an improved model of the pathway. The focus here lied on assembling new components into the given model. For this purpose a comparison of the models of Cho etal [1] and Schoeberl etal [2] was performed. Further more the model was checked against the interaction-map presented by Bouwmeester etal [3]. A sensitivity analysis regarding the initial values of the components was performed for both pathway models, the original one and the extended one. The matlab function sens_ind [4] was used to carry out this task.
Results The extended model encompasses three additional proteins: MEKK3, FLIP and TRAF1. All together the new model encompasses 8 additional components - three proteins and corresponding complexes. Further more five inconsistencies in the original model given by Cho etal [1] were eliminated. The sensitivity analysis shows that the behaviour of the extended pathway depends strongly on the initial values of the new added components.
Conclusion The sensitivity analysis of initial values showed that further investigations regarding the new model will be necessary. Challenge is a meaningful extension of the mathematical model. An open question to work on is, how to integrate new components and reactions into the model.
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M-P05 A domain-oriented approach to the reduction of combinatorial complexity in signal transduction networks

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Signaling networks play a crucial role in the regulation of a variety of cell functions. A common feature of signaling pathways is the formation of multiprotein signaling complexes. Receptors and scaffold proteins usually possess a number of distinct domains and bind multiple partners. The number of feasible multiprotein species grows exponentially with the number of binding domains and can reach thousands or even millions [1].
We introduce a systematic approach, which allows reducing signal transduction models considerably. A mechanistic description, which follows all possible states, is substituted by a macro-description. This approach can be compared with thermodynamics. At a microscopic level, a thermodynamic system should be described by position and speed of each molecule. However, in most cases it would be sufficient to know macroscopic properties like temperature, pressure and mass. The macro-states in our approach follow the levels of occupancy of binding domains [3,4]. These new quantitative indicators of the system (like degrees of phosphorylation) are widely used in biology and have a higher significance than the concentrations of each feasible multiprotein complex. This choice of states corresponds to the view that molecular domains, instead of molecules, are the fundamental elements of signal transduction networks [2]. In contrast to many other model reduction methods our approach is independent of numerical values. Qualitative biological knowledge about the domain-domain interactions is sufficient to derive the model equations.
Our method is based on the system theoretical concept of observability. Using a state space transformation, the complete mechanistic model can be transformed to new coordinates (including the levels of occupancy). Assuming that the levels of occupancy are the quantites of interest, the transformed model equations can be separated into observable states (states that influence the macro-states) and unobservable states (which can be neglected). Applying this method to the adaptor molecule LAT (Linker for activation of T-cells), the mechanistic model consisting of 36 differential equations could be reduced to a 10-state model [4].
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M-P06 Model building in a systems biology company: the cell cycle and apoptosis

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The work flow structure of the company with respect to model building will be discussed. This starts with literature research. It is difficult to obtain the necessary quantitative data from the literature. For this reason Physiomics plc has entered into a collaboration with the laboratory of Marta Cascante to obtain good quantitative data regarding a number of proteins involved in the cell cycle. Literature research is followed by the creation of detailed maps to aid model building. After this the programming to build the model takes place. The company uses Jarnac for single-cell modelling and proprietary software for modelling populations of cells. Then the model must be validated. This is done using experimental data based upon which experiments are carried out with the model. The model is then ready for use. The company's aim is to use models of biological systems to aid in drug discovery and development. The model was recently used successfully to test data regarding cyclin-dependent kinase inhibitors being developed as anti-cancer drugs. These processes can also be aided by physiology-based pharmacokinetic (PBPK) modelling using PK-Sim the software package Physiomics markets through a collaboration with Bayer Technology services. Recently literature research on apoptosis has been carried out in Physiomics with the aim of adding modelling of apoptosis to the cell cycle model. This research will be briefly discussed as well as the challenge of integrating modelling of apoptosis with the existing model of the cell cycle.

M-P07 Na,K-ATPase regulation via phospholemman phosphorylation

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Many hormones affect the Na^+, K^+ active transport, whose signaling pathways involve kinases. PKG has been shown to affect the Na,K-ATPase activity, however the mechanism is not known yet. Many attempts have been made to characterize the effects of protein kinases on Na,K-ATPase function, however, there is a lot of controversy since different researchers have found different (even opposite) effects. The regulation seems to strongly depend on the tissue under study, which led us to the idea that kinases may modulate Na,K-ATPase activity via intermediary proteins. Recent evidence shows that phospholemman (PLM), a single span transmembrane protein, can interact with Na,K-ATPase (1). PLM is expressed in several tissues where the Na pump is known to play an key regulatory role, such as heart and choroid plexus. PLM is known to be a substrate for several kinases, having at least two known phosphorylation sites. We propose that PLM could be the convergence point of different signaling pathways of regulation of the Na pump.
To determine the functional effect of the presence of PLM, we generated a PLM-knockout mouse (2). Heart sarcolemma from these mice had a much lower Na,K-ATPase activity than wild type, while the level of expression of the pump was only reduced by 15%. We have cloned and expressed human PLM in HEK cells, which have negligible endogenous expression of PLM. Membranes isolated from these cells were enriched in Na,K-ATPase by a procedure that removes many contaminating proteins. Na,K-ATPase activity was determined on this membranes as a function of Na concentration. Both V_{max} and K_{Na} were significantly different between cells expressing PLM and mock transfected controls. Incubation of choroid plexus membranes with ATP resulted in phosphorylation of PLM, which was blocked by a non-specific kinase inhibitor, suggesting the involvement of an endogenous membrane-bound kinase. PKA is known to phosphorylate S68 and PKC to phosphorylate S63 and S68. We obtained constructs for PLM mutants that constitutively mimick phosphorylated states of these sites by replacing S residues by D residues (so far: S63D, S68D, S63A, S68A). We are expressing these mutants in HEK cells to characterize the effect of different states of phosphorylation on Na,K-ATPase activity.
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2 Li Guo J et al. (2004) Am. J. Physiol. Heart Circ. Physiol. In the press.

M-P08 System Properties of the Core Reactions of Apoptosis

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Summary We present one qualitative and two quantitative methods for discriminating biological models. Applying these to the apoptotic core reactions not only helps to identify a suitable model structure but enables several new and interesting insights into system properties and performance of this signal transduction pathway.
Introduction Apoptosis is an important physiological process crucially involved in the development and homeostasis of multicellular organisms. Although the major signaling pathways leading from the extrinsic induction to the execution of apoptosis have been unraveled during the past years, a detailed mechanistic understanding of the complex underlying network remains elusive. Previous modeling efforts focused on a descriptive behavior of large parts of the pathway using data derived from population studies. However, new data show that, within a single cell, the majority of caspases is activated much faster than in cell populations.
Results Based on the current literature, we derive a differential equation based model for the direct signal transduction pathway of receptor induced apoptosis [1]. The new single cell data together with physiologically motivated, theoretical considerations state a requirement for a bistable behavior as a qualitative system property.
Bifurcation analyses show that bistability is only possible for parameter values far away from those reported in literature, indicating the presence of an additional control mechanism. We propose a new model with a suitable additional control structure. This extended model displays a bistable behavior and a fast caspase activation with kinetic parameters close to those reported in literature.
Employing these two models as test cases, we present two quantitative procedures for model discrimination based on the concept of robustness of biological behavior. Taking into account the non-linearity of the systems, the first method assesses the robustness of the bistable behavior with respect to parameter variations. The second method evaluates the robustness of the bistable threshold under the influence of the stochastic nature of reactions. These discrimination criteria favor the extended model, in accordance with our previous findings and novel experimental evidences.
Using a distributed input also allows us to reconcile the differences between the observed kinetics of single cells and populations in terms of understanding and modeling.
[1] Eißing et al. J. Biol. Chem. ***279****(35):36892-7.*

M-P09 Meshfree modelling of biological transport processes in complex domains

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Spatial models of biological systems often require the representation of a hierarchy of nested domains that can exhibit high geometrical complexity. Moreover, dynamical changes of geometrical properties are common.
We are interested in analysing, modelling and simulating passive or mediated transport processes in cells and organelles. The focus lies on complex interactions between compartments, i.e. domains and subdomains exchange substrates through their membranes, depending on the presence of certain receptors and signals. Interface characterestics can likewise be connected to specific processes like concentrations and gradients and change accordingly.
Systems of second order PDEs are used for describing the transport processes under investigation. For the special requirements of biological models, classic discretisations, based on a triangulation of the computational domain, often cause technical problems, which esp. is true in 3D. Our research is based on recent results in the theory of so called meshfree methods. This versatile approach has gained much attention in the engineering community during the last years for providing more flexible approximation space construction and nice adaptivity properties. We strive to adopt and extend a class of meshfree methods to make it applicable to the simulation of spatio-temporal processes in complex, nested domains.
One application is the simulation of a specific protein translocation pathway in the thylakoid, which is the organelle in the plant cell's chloroplast where photosynthesis takes place.

M-P10 Generating conceptual models in Zebrafish zinc homeostasis: The first steps towards an holistic view of zinc metabolism

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Zinc is an essential metal ion, utilised by over 3000 proteins and therefore zinc homeostatic control is an intrinsic requirement for every metabolic pathway. Characterising the complex adaptive control systems involved in zinc regulation, together with conceptual modelling of the potential metabolic sequelae and their feedback pathways demands a Systems Biology approach.
To date little work has been done to tie the role of individual zinc transporters with the compartmental models of whole body zinc homeostasis, not least because the epithelial sites of zinc exchange, the intestine, kidney and biliary system, are not readily accessible for detailed characterisation. In the teleost fish the gill is also a site of zinc exchange and this easily accessible epithelial surface presents an ideal experimentally manipulatable site for the clean and direct exploration of zinc handling in vivo without confounding effects. Building on the bedrock of physiological observations and mathematical modelling of zinc homeostasis in teleost fish and humans, we are using multiple approaches to inform the generation of conceptual models for zinc homeostasis in the zebrafish:
1) Using molecular biology combined with comparative bioinformatics and genome mining, we have identified more than twenty putative zebrafish zinc transporters each with its own profile of tissue-specific expression and zinc-regulation. Parallel experiments in cell culture have allowed the comparison of epithelial and non-epithelial zinc handling.
2) Specific functional knockdown of the metal responsive transcription factor MTF-1 mRNA using siRNA is revealing the role of this key zinc-regulator in the transcriptome responses to changing zinc conditions.
3) Microarray characterisation of zinc responses inform the tentative models of zinc homeostasis, allow the clear identification of novel zinc pathway genes and provide a genome-wide view of the transcriptional response to zinc excess and deficit.
Our Systems Biology approach has facilitated the formation of tentative tissue-specific models for zinc handling that will be combined to form a conceptual model for whole fish zinc homeostasis. In time such an approach will facilitate the simulation of the effects of aquatic zinc pollution in fish. Importantly, our approach is revealing the fundamental biology behind the complex, essential and sometimes novel mechanisms in place to ensure that sufficient zinc is supplied to meet metabolic requirements.

M-P11 Repression of SOX6 transcriptional activity by SUMO modification

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SOX6, a member of the SOX family of Sry-type HMG transcription factors, plays key functions in several developmental processes, including neurogenesis, sex determination and skeleton formation. In this report, we show that SOX6 is covalently modified in vitro and in vivo by SUMO1, 2 and 3 on two consensus sites (IK364NE and VK377DE). Mutation of both lysines to arginine abolished SOX6 sumoylation and increased SOX6 transcriptional activity as well as SOX6/SOX9 synergistic activation of the Col2a1 enhancer. SUMO dependent repression of SOX6 transcription was demonstrated by Ubc9 overexpression whereas siRNA to Ubc9, cotransfection of a catalytically inactive UBC9 or a SUMO specific protease increased SOX6 transcriptional activity. Immunofluorescence analysis showed a predominant diffused nuclear localization of SOX6 when expressed alone. Coexpression of SOX6 with SUMO1 and/or SUMO2 results in the appearance of SOX6 in a punctate nuclear pattern that colocalized with PML. PML body localization of SOX6 was abolished by mutations in SOX6 sumoylation sites. Thus, SUMO modification of SOX6 alters its subnuclear localization and leads to transcriptional repression.
ACKNOWLEDGEMENTS
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M-P12 Network synchronization from population to cell level

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Expression profiles prepared from cell cultures and tissues, are used to infer molecular networks underlying biological functioning. Assuming that each cell instantiates a temporal version of the same functional network, expression profiles measures a mixture over time of this network according to cellular dynamics. To address these issues in network engineering, we studied the relations when scaling models from individual to population cell levels.
In one individual cell, the list of RNA concentrations is a function x(t). An expression profile made at time T will involve a population of cells, and because of the divergent evolution of individual cells over time, the RNA content at the population level won't exhibit the dynamic of the single functional network.
As an example, we modeled the list of ages of cells as random variables, with common distribution. Under some conditions, we show that RNA concentration is approaching a quantity X(T) that is the image of x(t) by an integral operator.
Whatever the vector X(T) still represents a list of concentrations, it does not behave as the vector x(t), and in some bad cases, the measurements can’t be used to infer the network directly. We must then identify the kernel that define the integral operator. But modeling it appears to be a hard task, as the phenomenon of desynchronization inside a population of cell seems to be unreachable. Nevertheless, under some conditions, we can avoid this step, and directly build approximations of the initial curves of concentrations x(t). Generalizing the model, we propose a numerical method, which could also lead to the design of specific experiments.

M-P13 Impaired gene expression in Sjogren's disease

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Sjogren’s disease (SjS) involves salivary glands malfunctioning and disrupted cholinergic signaling. Here, we report SjS-associated changes in the acetylcholine (ACh) hydrolyzing enzyme, acetylcholinesterase (AChE) in acinar cells and secretory ducts from SjS salivary glands. Both AChE mRNA and interleukin 1 (IL-1) levels were reduced in acinar gland cells, suggesting an accompanying immunomodulary decline. In contrast, secretory ducts showed increased AChE expression and elevated IL-1 levels, demonstrating an inflammatory reaction associated with reduced ACh signaling and relieved suppression of proinflammatory cytokines production. Increasing severity of disease symptoms further involved AChE increases in acinar cells and residing lymphocytes and larger fractions of acini with polarized AChE-R mRNA distribution. To test relevance of AChE distribution for ACh metabolism, we used salivary gland sections from transgenic mouse lines overexpressing distinct splice variants of human AChE yet with similar ACh hydrolytic activities. Significant increases in the fraction of duct, but not acinar cells expressing nuclear “synaptic” AChE-S were found in TgR mice with excess of the stress-induced hAChE-R protein. Significant IL-1 decreases ,down to 60+6% of FVB/N controls, occurred in acinar, but not duct cells, of TgR mice ($p<0.001$, Student’s t-test) as well as in TgS mice overexpressing human AChE-S and murine AChE-R ($p<0.005$). TgSin mice expressing genetically inactivated AChE-S presented a trend for further IL-1 decline ($p>0.3$), altogether suggesting causal involvement of cholinergic signaling. Our findings suggest contribution of the stress-inducible AChE-R splice variant to the initiation and progression of the compromised secretory features and immune profile of salivary glands from Sjogren’s disease patients.

M-P14 Modeling the synchronization of circadian oscillators in the suprachiasmatic nucleus

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In mammals, the suprachiasmatic nucleus (SCN) constitutes the central pacemaker which controls circadian rhythms in physiology and behavior. Individual SCN cells exhibit sustained circadian oscillations with periods ranging from 20 to 28 h. These oscillations are generated by a molecular regulatory network based on a negative feedback loop. On the tissue level, SCN neurons display a significant degree of synchrony. Neurotransmitters have been shown to play a crucial role in the coupling mechanism. Depending on the type of neurotransmitters released by the cells, the SCN has been subdivided into two regions, the ventro-lateral and the dorso-medial parts. Only the neurons present in the first region are sensitive to light and convey the light signal to the dorso-medial region. Furthermore when isolated from the ventrolateral part, dorso-medial cells get out of phase. However, unexpectedly, the dorso-medial part is phase leading. We present a mathematical model for the coupling of a population of ten thousand circadian oscillators in the SCN. The cellular core oscillator is described by a three-variable model relying on a negative feedback loop. The coupling is incorporated through the global level of the neurotransmitter concentration. We first show that such a global coupling is efficient to synchronize a population of thousands cells. The synchronized cells can be entrained by a 24 h light-dark cycle. The study of the interaction between two population of oscillators, representing the two regions of the SCN, shows that the driven region can be phase leading. An experimentally testable prediction of our model is that synchrony is reached when the average neurotransmitter concentration brings the cells outside their region of individual sustained oscillation.

M-P15 Modelling, Enzyme kinetics & Fluorescence Imaging of the NF-kappaB Signalling Pathway

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Analysis of cellular signalling interactions is expected to create an enormous informatics challenge, perhaps even greater than that of analysing the genome. We have reconstructed a model of the NF-kappaB signalling pathway, containing 64 parameters and 26 variables, including steps in which the activation of the nuclear factor kappaB (NF-kappaB) transcription factor is intimately associated with the phosphorylation and ubiquitination of its inhibitor kappaB by a membrane-associated kinase, and its translocation from the cytoplasm to the nucleus. We apply sensitivity analysis to the model. This identifies those parameters in this IkappaB-NF-kappaB signalling system (containing only induced IkappaBalpha isoform) that most affect the oscillatory concentration of nuclear NF-kappaB (in terms of both period and amplitude). We have also measured enzyme kinetics of IKK2 with the substrates in this pathway and have studied the impact of these data in the model. We observed these new data to have a profound effect on the model. We also carried out fluorescence cell-based imaging and studied the correlation of measured enzyme kinetics with the observed images.

Ihekwaba, A. E. C., et al. (2004). Sensitivity analysis of parameters controlling oscillatory signalling in the NF-kappaB pathway: the roles of IKK and IkappaBalpha. Syst. Biol. 1, 93
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M-P16 Towards a systems biology of signal transduction by insulin and insulin-like growth factors.

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Insulin and the related insulin-like growth factors exert their effects by binding to and activating separate but related membrane receptor tyrosine kinases, triggering a complex intracellular network of signaling pathways. Disorders of these systems lead to serious diseases like diabetes, metabolic syndrome, small size and cancer. Physiological and genetic evidence suggests that insulin is primarily a metabolic regulator, while the related IGF-I and IGF-II are primarily growth promoters. Despite extensive studies, the molecular basis of this specificity of actions is still poorly understood, because:
- the ligands as well as the receptors are closely related
- their signaling network are largely overlapping
- each receptor has been shown to be able to mediate the effects of each ligand in a given cellular context.
It is clear that new methodological approaches that include cellular imaging, real-time kinetic analysis and network integrated analyses are required to progress in understanding the combinatorial nature of signaling specificity.
Our approach to this problem consists in combining multiple novel methodologies:
• Real-time kinetic measurements of receptors and proximal signalling molecules (IRS 1-6, PTP-1B) using FRET (fluorescence resonance energy transfer) and BRET (bioluminescence resonance energy transfer).
• Confocal microscopy for live cellular imaging of signalling events using fluorescent probes.
• Microarray gene profiling.
• siRNA interference with key signalling proteins.
Target cells explored include human preadipocytes, a rat beta cell line, human myoblasts from normal and diabetic subjects, myoblasts from insulin receptor knockout mice, and mouse cell lines devoid of IGF-II/M6P receptors.
The ultimate goal is to reverse engineer end point biological data and correlate them by mathematical modelling with the kinetic aspects of receptor binding of IGF-I and II as well as insulin analogues with different kinetics, and real-time measurement of key signalling steps.
This program is supported by the BIO+IT programme of Øresund IT Academy, Medicon Valley Academy, and the Danish Ministry of Science, Technology and Innovation.

M-P17 BOOLEAN analysis of the signaling network triggered by neurotrophic factors and extracellular matrix in sensory neurons

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Neurotrophic factors and laminins are important regulators of posttraumatic regeneration in the nervous system. Cyclin-dependent kinase 5 (Cdk5) regulates cytoskeleton mobility and mediates the effect of neurotrophic factors on axonal growth in various types of neurons. Here we show that NGF, GDNF and neurturin activate axonal growth in mature dorsal root ganglion neurons in the absence of Cdk5 activity as the effect of these neurotrophic factors was not affected by 50uM of roscovitine. On contrary laminin-dependent outgrowth in the absence of neurotrophic factors was fully blocked by roscovitine. GDNF- and laminin-dependent types of axonal growth also have different sensitivity to src inhibitor SU6656. We use Boolean networks formalism to analyze differential contribution of neurotrophic factors- and laminin-triggered pathways to the converging signaling network.

M-P18 A topological analysis of the human transcription factor interacting network

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Protein-protein interactions are one of the bases of regulation in biosignalling and gene expression regulation. Massive biological data acquisition is allowing to study biological systems as whole. For this purpose, it is necessary the use of systemic approaches, such as is the case of graph theory. In this context, several protein interacting networks or so called interactomes have been described in the last years.
In this work, we study the human protein-protein interacting transcription factor network, obtained from data contained in a transcription database (Transfac), using graph theory.
Clustering coefficient and diameter of the network show a small-world pattern and distributions for degree, betwenness centrality and clustering suggested a scale-free behaviour, as it is occurs with other biological networks. Topological overlap matrix and correlation profiles were calculated and we could see that this transcriptional network is also hierarchical and shows modularity. Modules are composed of factors with both functional, structural features. Some modules include factors that share function but not structural features. However, others showed similar structure and common functionality or, indeed, phylogenetic origins. According to these data, we suggest that two motors have modelled the transcription network. The first one would be based on gene amplification and shuffling of certain domains such as bHLH and Zn fingers motifs, capable to form dimers allowing for the interaction of proteins, most probably due to the necessity of new regulatory factors during multicellularity acquisition in the evolution. On the other hand, proteins without these general binding domains could establish connections by a random process yielding some benefit for the system. Some features of this network can be derived from biological and structural constrains, such as the grade of autolinks explained by the use of these kind of domains that allow a regulation with a low cost of structure types.
We also show that it is possible to identify potential relevant elements in the system using topological properties. For instance most of highly-connected nodes are related with proliferative processes. On the other hand, nodes with few connections and high betweenness centrality levels could be important due to the neighbours they connect.

We conclude that using topological approaches for the study of a not yet wholly-described system can give relevant information about the nature of gene regulation.

M-P19 Molecular dissection of the key LGS residues involved in the control of glycogen biosynthesis.

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Glycogen synthase (GS) catalyses the addition of glucose residues to the non-reducing end of a nascent glycogen chain via alpha-1,4-glycosidic bonds, using UDP-glucose as substrate. Two isoforms of mammalian GS have been described; most tissues express the muscle form, whereas the liver isoenzyme (LGS) appears to be tissue-specific. GS activity is highly regulated via phosphorylation and allosteric effectors, mainly glucose 6-phosphate (Glc-6-P).
It is generally accepted that the reaction catalysed by GS is rate-limiting for glycogen synthesis in all organs [1]. The importance of this enzyme in the overall process of glycogen deposition is confirmed by the observation that overexpression of GS in cultured hepatocytes increases glycogen accumulation [2]. This is the consequence of the action of Glc-6-P produced by endogenous glucokinase (GK). This metabolite causes the allosteric activation of the total amount of LGS, through a conformational rearrangement that converts this enzyme into a better substrate for protein phosphatases, which catalyze its dephosphorylation, thus leading to an increase of 'active' LGS. Moreover, when GK is overexpressed, the increase in Glc-6-P results in a higher degree of activation of the endogenous GS, which also leads to the deposition of larger amounts of glycogen. Finally, when both enzymes are overexpressed, there is a combination of the two effects. Therefore GK and LGS share the control of hepatic glycogen biosynthesis, in which the control exerted by LGS is in turn controlled by GK.
We aim to make the molecular dissection of the key LGS residues involved in the control of glycogen biosynthesis. To this end, we are currently generating adenoviruses of LGS where Ser to Ala mutations have been introduced individually or in combination at phosphorylation sites of the enzyme. These adenoviruses will provide further insight into the role of GS and GK in the control of LGS.
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M-P20 Analysis of the signaling network involved in the activation of T-Lymphocytes

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Despite the intense research and the considerable progress within the last years, how T-lymphocytes are able to distinguish foreign agents among the myriads of components of our own body is still not fully understood. T-cell reactivity has to be exquisitely regulated since not only a decrease (since it weakens the defense against pathogens), but also an increase (which can lead to autoimmune disorders) can be dangerous.
The central sensor in the recognition process is the T-Cell receptor (TCR). Upon binding of an antigen to the TCR, several signaling processes take place. Additionally to the ligand itself, other signals from other cells of the immune system are also sensed by different receptors on the T-cell membrane. The resulting signaling network, of extreme complexity, assures that T-cells become activated only when and where they should.
We try to unravel this complexity by a combination of analyses at different levels and with different tools. On one hand, we perform a qualitative analysis of an extensive portion of the network. On the other hand, we concentrate on a key process, namely the activation of the MAPK Cascade and its dynamics. For this pathway, a detailed mechanistic model is set up. The model is fitted by quantitative measurements of activation levels of key proteins performed on naive T-cells from transgenic mice. The experiments will also be extended to cases where the network is perturbated (e.g. by knock-out mice, inhibitors, iRNA, etc.).
We try to address several key questions, such as the importance of the antigen:TCR affinity, the topology of the TCR signal transduction network -in particular feedbacks mechanisms (Reth, M., et al., Nat Rev Immunol.,(2004), 4(4):269),- and the role of transmembrane adaptor proteins like PAG (Horejsi, V., et al. Nat. Rev. Immunol., (2004), 4(8):603). For this purpose the model is decomposed into modules connected unidirectionally, which are subsequently analyzed. Rewiring together the modules new insights into the whole cascade can be obtained (*Saez-Rodriguez, J., et al., IEEE CSM, (2004), 24(4):35*).

M-P21 Flavo-di-iron proteins: role in microbial detoxification by NO

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The flavo diiron proteins (FDP) are soluble flavoproteins discovered in facultative and strict anaerobic prokaryotes. They are built by the two core domain: a metallo beta-lactamase domain at the N-terminal region harboring a non heme di-iron site and a flavodoxin-like domain. Recently it has been proposed that this proteins have a role in NO metabolism [1]. Evidence for NO reductase activity was provided by amperometric measurements on the ricombinant isolated enzyme from *Escherichia coli* [Gomes]. It has been generally believed that FDPs were restricted to the Archea and Eubacteria domains, until genomic analysis [2] demonstrated that microaerobic pathogenic protists (like *Giardia lamblia, Entamoeba histolytica* and *Spironucleus barkhanus*) have genes coding for FDPs. The working hypothesis is that via lateral gene transer these pathogenic mireoorganism acquired NO-reductase activity to counteract tha microbicidal action of NO produced by the host macrophages.
We have direct evidence that the microaerbic eukaryotic parasite *Trichomonas vaginalis*, the causative agent of trichomoniasis which seems to have at least three genes coding tor FDP, degrades NO under anaerobic conditions [Sarti]. The NO degradation activity is: maximal at low NO concentraton, NADH-dependent, cyanide insensitive and is inhibited by O_2. In addition a protein band was immunodetect using antibodies again the *E. coli* FDP. This acquired NO reductase activity might be at the basis of mechanism of defense which helps *T. vaginalis* to survive at high fluxes of NO produced by immune response of human host.
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M-P22 Quantitative modeling of EGFR-internalization as a mechanism of signaling specificity

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Epidermal Growth Factor binding to its receptor (EGFR) results in a variety of cellular responses, communicated by phosphorylation cascades via receptor tyrosine kinases (RTKs) and ultimate activation of transcription factors like Myc, Fos and Elk. Signal attenuation is achieved by receptor internalization to endosomes and eventual degradation. However, there is growing evidence that intracellular trafficking to endosomes itself constitutes a mechanism of regulating signal specificity. Suppression of endocytosis does not lead to a uniform up-regulation of EGF-dependent signaling events and numerous RTK-specific regulators affecting endosome sorting have been identified [1].
We have developed a quantitative mathematical model, which explicitly incorporates intermediate steps of transport and degradation of internalized receptor. Specific key questions the model addresses are: Which are the crucial features of receptor endocytosis mediating signal specificity? Which are the critical parameters in the system that determine the fate of internalized EGFR? The existence of phosphatase gradients in the cytosol has been observed [4], our model investigates whether signaling endosomes are used to overcome these phosphatase barriers and thus propagating the signal inside the cell.
Model development is driven by in vivo data retrieved from fluorescence microscopy images on living cells [2]. Here, the phospohrylation state of EGFR and downstream effectors such as Mek, Ras and Erk have been imaged under conditions perturbating endocytosis and down-regulated phosphatase activity by measuring fluorescence resonance energy transfer.
In order to deal with the problem of underdetermined systems, we are using a sensitivity analysis of key parameters previously employed in our lab [3], which reduces the effective dimension of the model by, e.g. identifying correlated parameters.
Based on the model we have developed a simulation software, which performs parameter estimation using fluorescence images.
We believe that a *quantitative* analysis of this pathway will lead to new insights in this important pathway, since it is well established by now that kinetic differences lead to very different cellular behaviour.
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M-P23 Retroelement insertion polymorphism in cell line identification.

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Cell lines are an indispensable, renewable resource for numerous studies in various fields of modern biology and medicine. It is assumed that the cell features in a particular cell line are similar with that in the human tissue from which this line originated. However, most human cell lines are prone to various sorts of genetic rearrangements that affect biochemical, regulatory and other phenotypic features of cells during their cultivation. Various chromosomal abnormalities including aneuploidy, numerous rearrangements and loss of chromosome regions are characteristic alterations of cell genomes, especially if the cells are of tumor origin. Therefore, it is highly desirable to permanently monitor the authenticity of the cell lines and/or to have reliable cell line identification techniques to make sure that the cell lines to be used in experiments are exactly what is expected. To this end, we developed a set of informative markers based on insertion polymorphism of human retroelements. The set includes 47 pairs of PCR primers corresponding to introns of the human genes with dimorphic L1 and Alu insertions. Using locus specific PCR assay, we have genotyped 10 human cell lines of various origin. For each of these cell lines characteristic fingerprints were obtained. An estimated probability that two different cell lines possess the same marker genotype is about 10^{-18}. Therefore, the proposed set of markers provides a reliable tool for cell line identification.

M-P24 RNAi screening for novel components of mammalian Hedgehog and Wnt pathways

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RNA-mediated interference (RNAi) is a powerful reverse genetic tool to silence gene expression in organisms ranging from plants to humans. The initial finding that siRNAs and constructs encoding short hairpin RNAs (shRNAs) could trigger gene silencing in mammals has been refined and extended by the combination of shRNA with viral and episomal vectors that allow transient or stable silencing in mammals (1). This has encouraged and broadened interest in using mammalian cells for both forward and reverse genetic screens.
In this study we tested the feasibility of two different vector-based Gateway-compatible RNAi systems (siRNA and shRNA) by analyzing their knockdown efficiency by measuring the ability of these vectors to specifically suppress target gene –Renilla-luciferase fusion proteins. In our hands, the shRNA vectors had much greater efficiency in this assay. We next validated the shRNA system by testing the ability of co-transfected shRNAs targeting known components of the Hedgehog or Wnt signaling pathway to inhibit luciferase-based Hh or Wnt signaling reporters, respectively. The luminescence based signalling assays allows us to analyze the effect of knockdown of different genes in high-troughput manner in cultured cells. To identify novel effectors of these pathways, we designed shRNAs targeting nearly all mouse kinases using thermodynamic criteria (2), and genereted the corresponding arrayed shRNA-library. Using this vector-based shRNA-library, we show that transfected as well as endogenous genes can be efficiently inhibited. We are currently using this library to identify new components of mammalian Hedgehog and Wnt signaling pathways. These findings highlight the general utility of this vector-based RNAi technology in suppressing gene expression in mammalian cells in high-throughput and cost-efficient manner.
1. Paddison PJ & Hannon GJ. (2003) Curr Opin Mol Ther. 5(3):217-24
2. Schwarz, D. S. et al. (2003) Cell 115, 199-208

M-P25 Modeling emergent networks by dynamic reconstruction *in silico*

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Molecular interactions in cells of metazoans are context dependent, with rich semantics, evolve with cell fate specification, reprogrammable, and create emergent networks. To unveil the dynamic process of how signaling and mis-signaling among molecules leads to patterning and mis-patterning, functioning and mis-functioning of cells, modeling and simulation that couples intra- and inter-cellular, molecular and cellular events are required to systematically probe the evolution of networks and the order and timing of signaling. We advocate a modeling paradigm and introduce a modeling platform, which are based on the combining of cellular automata parallelism and object-oriented programming. The proposed method features: (1) object-oriented extensions to a language-based cellular automaton to create a two-tier parallelism; (2) event-driven signaling; (3) flexible qualitative and quantitative computation; (4) dynamic capture and display of signaling events and molecular attributes; (5) dynamic reconstruction of signaling networks. The cell-object(molecule) two-tier structure enables the modeling of parallel molecular networks in cells; and the separation of discrete signaling simulated as message passing among objects, from continuous computation in the form of ODE and so on, allows a realistic description of large scale systems. The dynamic captured signaling explains both the evolution of networks and the order and timing of signaling in cells. With an exemplary 2D model on mouse somite segmentation, we exhibit that networks show dynamics and evolve with cell fate specification. Complex, including seemingly chaotic, global behaviors can be well elucidated with the captured signaling events. In addition to the partnership of molecular interactions which determines network topology, we find the order and timing of signaling, termed as *network dynamics* here, are also crucial. Signaling at wrong time and in wrong order may explain a large group of anomalies, which may not necessarily have a wrong molecular interaction topology. An ectopic or unduly signal may fundamentally change the signaling inside a cell, and thus the fate of the cell, as widely observed in many cell fate transformation events. These factors often mingle together context dependently, making signaling emergent and elusive and demanding effective computational simulation.

Power posters

M-PoP1 **Niels Aarsaether**

M-PoP2 SYMBIONIC: A European initiative on the Systems Biology of the neuronal cell

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No abstract submitted.

M-PoP3 *In vitro* systems for modelling of signal transduction in hepatocytes

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The aim of our consortium is to model specific signal transduction pathways as well as detoxification processes in hepatocytes. For this purpose, standardized in vitro systems with hepatocytes are needed. We established standardized hepatocyte systems including (i) cultured primary human and mouse hepatocytes, (ii) stem cell derived hepatocyte like cells, including hepatopancreatic precursor cells that have been conditionally immortalizeed by Bmi-1 and hTERT, (iii) constitutively as well as conditionally immortalized adult and fetal hepatocytes.
TGF-b participates in several aspects of liver damage, including hepatocyte apoptosis. We established tools to monitor TGF-b signalling in murine and human hepatocytes. Functional ectopic protein expression of YFP and CFP fusion proteins for TGF-b type I (ALK5) and type II receptors was confirmed biochemically and with confocal microscopy. The constructs will be used with the Opera System (Evotec) to study endocytotic trafficking of the receptor complex during signalling (TGF-b treatment) and degradation processes (Smad7/Smurf1). TGF-b signalling was documented by phosphorylation of Smad2, activation of a Smad binding element containing reporter construct, (CAGA)9-MLP-Luc, and by triggering apoptosis, presented as annexin staining or DNA laddering assay. To interfere with specific steps of TGF-b signalling in hepatocytes, we used dominant negative TGF-b receptors, Alk5 inhibitor SB 431542, Smad7, Ski and SnoN. For modelling purposes, TGF-b receptor complex cargo will be monitored optically with the Opera based on time course experiments after ligand binding and/or after the above mentioned treatments.

M-PoP4 Module dynamics of the GnRH signal transduction network

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We analyze computational modules of a frequency decoding signal transduction network. The gonadotropin releasing hormone (GnRH) signal transduction network mediates the biosynthesis and release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). The pulsatile pattern of GnRH production by the hypothalamus has a critical influence on the release and synthesis of gonadotropins in the pituitary. In humans, slower pulses lead to the expression of the beta subunit of the LH protein and cause anovulation and amenorrhea. Higher frequency pulses lead to expression of the alpha subunit and a hypogonadal state. The frequency sensitivity is a consequence of the structure of the GnRH signal transduction network. We analyze individual components of this network, organized into three network architectures, and describe the frequency-decoding capabilities of each of these modules. We find that these modules are comparable to simple circuit elements, some of which integrate and others which perform as frequency sensitive filters. We propose that the cell computes by exploiting variation in the time scales of gene activation (phosphorylation) and gene expression.

M-PoP5 Experimental design for model discrimination in cellular signal transduction

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Dynamic modeling of signal transduction pathways helps to understand cellular processes. This systems biology approach requires quantitative, time-resolved measurements of the participating molecules, which are usually expensive and time consuming. Hence, experiments should be designed in advance being capable to (a) specify the correct model and (b) determine the kinetic parameters with a minimal effort. Several methods are proposed specifying the optimal experimental design in order to estimate the kinetic parameters, given a mechanistic model. However, often no explicit model exists for a biochemical system or serveral models are suggested and the question arises, which model describes the reality most accurately. We propose a new approach to determine the optimal experimental design addressing model selection. We defined a *model sensitivity*, a measure for the dependency of the dynamics of a protein concentration on the model structure. The model sensitivity can be used for ranking the most important system players which should be measured to discriminate models and identifies a minimal combination of measured proteins for a significant model discrimination.
Given this combination of proteins, the sampling time of the measurement is optimized to improve furthermore the expected significance of the model selection procedure.
As an example, two different models of the MAP-kinase signalling cascade are investigated. We identified the minimum set of necessary measurements to distinguish both models.

M-PoP6 Integration of genomics and proteomics with metabolic/signaling pathways for generating/improving novel anti-cancer drug targets

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cDNA microarray has become a powerful tool for profiling differential gene expression of cancer cells versus normal cells. With extraction of marker genes based on cDNA microarray data, we can better understand the regulatory genes, leading to improvement of drug targeting. A direct suggestion in drug targeting can come from differentially expressed proteins obtained from comparative proteomic data. A complete metabolic/signaling pathway depicts the interaction mechanisms among metabolites (or proteins) and proteins (or genes) and thus can also be a powerful tool for designing anti-cancer drug targets. It seems that any of these methods (genomics, proteomics and metabolic/signaling pathways) could be individually used for finding the drug targets for curing cancers. However, all these methods are currently incomplete. Genomics and proteomics data will produce a false discovery rate of marker genes and proteins, while metabolic/signaling pathways are most likely incomplete due to the lack of thorough understanding of cancer development. In this project, gene and protein expression data are used to check the correctness of a pathway from a component A to another combination B. Furthermore, these data are employed to determine whether there is an additional hidden component C between the components A and B. The likelihood of the existence of such a component will also be estimated. Such integration of genomic and proteomic data into the pathway leads to construction of a better pathway model and thus improved drug targeting.

Additional Teacher Abstracts

Looking for new targets in cancer therapy from a Systems Biology Approach

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In the last few years, science has concentrated their efforts in characterizing the molecular elements that conform cells. 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. Moreover, the integration of all these information in in vivo models is likely to be the most interesting tool to understand and to complete an overview picture of the cellular processes. We must take into account, that metabolic profile is in most cases the end point of the signalling events, where changes caused by diseases like cancer may be reflected. Therefore, using integrative bioinformatics tools we are able to identify the main steps that control a metabolic pathway after the integration of all the experimental data. These control points may be used as new therapeutical targets. In this way, we are working in new antitumoral treatments based on the inhibition of the synthesis of DNA and RNA because of their importance for cell proliferation. We have focused on ribose-5-phosphate synthesis, which is a component of nucleotides. First of all, we characterized the metabolic pathways (utilizing gas chromatography coupled to mass spectrometry) implied in glucose metabolism and ribose synthesis. This was followed by the integration of the obtained data in mathematical models which led us to identify the main enzymes controlling ribose-5-P synthesis: transketolase and glucose-6-phosphate dehydrogenase. Finally, we validated the obtained targets using specific inhibitors and then, we studied the effects produced in cell proliferation as well as in the proteomic profile. Consequences of the utilization of these inhibitors on protein-protein interactions and on supramolecular organization of the tumor metabolism have also been studied.

Mathematical modeling of mammalian nucleotide excision repair based on *in vivo*

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Nucleotide excision repair in mammals requires the concerted action of many different proteins. The repair machinery assembles itself at damaged-DNA sites in a strict sequential fashion. We have developed methods to visualize and quantitatively analyse this process in the nucleus of the living cell (Moné et al, 2001, 2004). Based on our measurements we constructed a mathematical model that delineates hallmarks and general characteristics for this repair pathway. These data allowed us to scrutinize the dynamic behavior of the nucleotide excision repair process in detail. The strict sequential assembly mechanism appears to be remarkably advantageous in terms of repair efficiency. Our findings show that alternative mechanisms, for instance the forming of repair protein complexes in the nucleoplasm before binding to damaged DNA, or random sequence of protein assembly, can readily become kinetically unfavorable. Our model provides a kinetic framework for nucleotide excision repair and rationalizes why many multiprotein processes within the cell nucleus may opt for a strategy of sequential assembly. The model constitutes a firm basis for further wet experiments. *Moné, M.J. et al. (2001) EMBO Reports. 2:1013*
Moné, M.J. et al. (2004) Proc Natl Acad Sci U S A. 101:15933

Principles of Systems Biology: the chapter on signal transduction

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Systems Biology is not only about the analysis of massive datasets, or the modelling of complex networks. Life follows certain principles. These derive from constraints such as steady state, as well as a number of fundamental properties of living systems. In this presentation we shall refer to principles such as that every process in a living cell is catalyzed by a protein, and that every protein is encoded by a (set of) gene(s). These principles are wrong if applied strictly to certain pathways, close to reality for others but may serve as a source of inspiration for all. They induce one to ask about the extent to which biological functions are controlled by the process activities.
It is this inspiration also that induced us to ask what the control of the total genome, i.e. of all activities combined, is on biological functions such as steady state flux or the duration of a signal. We adress this question by summing the control exercised by the individual processes, over all processes. Importantly then, well defined answers are obtained for certain functions. We here discuss the functional characteristics of signal transduction from this perspective.

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