BACTOCOMNEWS

August 2012

A periodic newsletter, reporting on the activities of the FP7-funded BACTOCOM project

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Continued success

Coordinator's report, Prof. Martyn Amos

BACTOCOM has continued its record of high-profile publications, with a number of notable papers published since the last newsletter.

We successfully negotiated our second year project review - see page 6 for a report.

In the profile section we introduce researcher Andrea Meyer, and highlight the work of advisory board member Jeff Hasty.

We also bring exciting news from various partners, including a significant award to the TUM team.

The project has been awarded a deadline extension of six months, which means the project will now finish at the end of July 2013. We are entering the critical integration phase of the project, so watch the website for further developments!

BACTOCOM contact

Prof. Martyn Amos
M.Amos@mmu.ac.uk
P: +44 (0)161 247 1534

www.bactocom.eu

Multi-target regulation by small RNAs synchronizes gene expression thresholds and may enhance ultrasensitive behavior

Jörn Matthias Schmiedel, Ilka Maria Axmann and Stefan Legewie

Cells respond to external cues by precisely coordinating multiple molecular events. Co-regulation may be established by the so-called single-input module (SIM), where a common regulator controls multiple targets. Using mathematical modeling, we compared the ability of SIM architectures to precisely coordinate protein levels despite environmental fluctuations and uncertainties in parameter values. We find that post-transcriptional co-regulation as exemplified by bacterial small RNAs (sRNAs) is particularly robust: sRNA-mediated regulation establishes highly synchronous gene expression thresholds for all mRNA targets without a need for fine-tuning of kinetic parameters. Our analyses reveal that the non-catalytic nature of sRNA action is essential for robust gene expression synchronization, and that sRNA sequestration effects underlie coupling of multiple mRNA pools. This principle also operates in the temporal regime, implying that sRNAs could robustly coordinate the kinetics of mRNA induction as well. Moreover, we observe that multi-target regulation by a small RNA can strongly enhance ultrasensitivity in mRNA expression when compared to the single-target case. Our findings may explain why bacterial small RNAs frequently coordinate all-or-none responses to cellular stress.

PLoS ONE 7(8): e42296. doi:10.1371/journal.pone.0042296

Probabilistic reasoning with a Bayesian DNA device based on strand displacement

Iñaki Sainz de Murieta and Alfonso Rodríguez-Patón

We present a computing model based on the DNA strand displacement technique which performs Bayesian inference. The model will take single stranded DNA as input data, representing the presence or absence of a specific molecular signal (evidence). The program logic encodes the prior probability of a disease and the conditional probability of a signal given the disease playing with a set of different DNA complexes and their ratios. When the input and program molecules interact, they release a different pair of single stranded DNA species whose relative proportion represents the application of Bayes' Law: the conditional probability of the disease given the signal. The models presented in this paper can empower the application of probabilistic reasoning in genetic diagnosis in vitro.

In Stefanovic, D. and Turberfield, A. (Eds.), Lecture Notes in Computer Science, 7433, DNA Computing and Molecular Programming, 110-122. doi: 10.1007/978-3-642-32208-2

Fine-tuning tomato agronomic properties by computational genome redesign

Javier Carrera, Asun Fernández del Carmen, Rafael Fernández-Muñoz, Jose Luis Rambla, Clara Pons, Alfonso Jaramillo, Santiago F. Elena and Antonio Granell

Considering cells as biofactories, we aimed to optimize its internal processes by using the same engineering principles that large industries are implementing nowadays: lean manufacturing. We have applied reverse engineering computational methods to transcriptomic, metabolomic and phenomic data obtained from a collection of tomato recombinant inbreed lines to formulate a kinetic and constraint-based model that efficiently describes the cellular metabolism from expression of a minimal core of genes. Based on predicted metabolic profiles, a close association with agronomic and organoleptic properties of the ripe fruit was revealed with high statistical confidence. Inspired in a synthetic biology approach, the model was used for exploring the landscape of all possible local transcriptional changes with the aim of engineering tomato fruits with fine-tuned biotechnological properties. The method was validated by the ability of the proposed genomes, engineered for modified desired agronomic traits, to recapitulate experimental correlations between associated metabolites.

PLoS Comput Biol 8(6): e1002528. doi: 10.1371/journal.pcbi.1002528

Synthetic in vitro transcription circuits

Maximilian Weitz and Friedrich C. Simmel

With the help of only two enzymes—an RNA polymerase and a ribonuclease—reduced versions of transcriptional regulatory circuits can be implemented in vitro. These circuits enable the emulation of naturally occurring biochemical networks, the exploration of biological circuit design principles and the biochemical implementation of powerful computational models

Transcription 3:2, 1-5. doi: 10.4161/trns.3.2.19734.

A degenerate primer MOB typing (DPMT) method to classify gamma-proteobacterial plasmids in clinical and environmental settings

Andrés Alvarado, M. Pilar Garcillán-Barcia and Fernando de la Cruz

Transmissible plasmids are responsible for the spread of genetic determinants, such as antibiotic resistance or virulence traits, causing a large ecological and epidemiological impact. Transmissible plasmids, either conjugative or mobilizable, have in common the presence of a relaxase gene. Relaxases were previously classified in six protein families according to their phylogeny. Degenerate primers hybridizing to coding sequences of conserved amino acid motifs were designed to amplify related relaxase genes from γ-Proteobacterial plasmids. Specificity and sensitivity of a selected set of 19 primer pairs were first tested using a collection of 33 reference relaxases, representing the diversity of γ-Proteobacterial plasmids. The validated set was then applied to the analysis of two plasmid collections obtained from clinical isolates. The relaxase screening method, which we call "Degenerate Primer MOB Typing" or DPMT, detected not only most known Inc/Rep groups, but also a plethora of plasmids not previously assigned to any Inc group or Rep-type.

PLoS ONE 7(7): e40438. doi: 10.1371/journal.pone.0040438

The diversity of cyanobacterial metabolism: genome analysis of multiple phototrophic microorganisms

Christian Beck, Henning Knoop, Ilka M Axmann and Ralf Steuer

Cyanobacteria are among the most abundant organisms on Earth and represent one of the oldest and most widespread clades known in modern phylogenetics. As the only known prokaryotes capable of oxygenic photosynthesis, cyanobacteria are considered to be a promising resource for renewable fuels and natural products. Our efforts to harness the sun's energy using cyanobacteria would greatly benefit from an increased understanding of the genomic diversity across multiple cyanobacterial strains. In this respect, the advent of novel sequencing techniques and the availability of several cyanobacterial genomes offers new opportunities for understanding microbial diversity and metabolic organization and evolution in diverse environments. Here, we report a whole genome comparison of multiple phototrophic cyanobacteria. We describe genetic diversity found within cyanobacterial genomes, specifically with respect to metabolic functionality. Our results are based on pair-wise comparison of protein sequences and concomitant construction of clusters of likely ortholog genes. We differentiate between core, shared and unique genes and show that the majority of genes are associated with a single genome. In contrast, genes with metabolic function are strongly overrepresented within the core genome that is common to all considered strains. The analysis of metabolic diversity within core carbon metabolism reveals parts of the metabolic networks that are highly conserved, as well as highly fragmented pathways. Our results have direct implications for resource allocation and further sequencing projects. It can be extrapolated that the number of newly identified genes still significantly increases with increasing number of new sequenced genomes. Furthermore, genome analysis of multiple phototrophic strains allows us to obtain a detailed picture of metabolic diversity that can serve as a starting point for biotechnological applications and automated metabolic reconstructions.

BMC Genomics 13:1, 56. doi: 10.1186/1471-2164-13-56.

Determination of conjugation rates on solid surfaces

Irene del Campoa, Raúl Ruiza, Ana Cuevasa, Carlos Revillaa, Luis Vielvab and Fernando de la Cruza

A cytometric method for the estimation of end-point conjugation rates is developed and adapted to surface conjugation. This method improves the through-put of conjugation assays based on replica-plating and results in less noisy experimental data. Although conjugation on solid surfaces deviates from ideal conditions in which cells are continuously mixed, results show that, within the limits of high initial population densities and short mating times, end-point estimates of the conjugation rates are robust measurements. They are independent of the donor/recipient ratios and, to some extent, of the sampling time. Remixing the mating population in the course of a conjugation experiment results in a boost in the frequency of transconjugants.

Plasmid 67:2, 174-182, doi: 0.1016/j.plasmid.2012.01.008.

Perspectives on the automatic design of regulatory systems for synthetic biology

Guillermo Rodrigo, Javier Carrera, Thomas E. Landrain and Alfonso Jaramillo

Automatic design is based on computational modeling and optimization methods to provide prototype designs to targeted problems in an unsupervised manner. For biological circuits, we need to produce quantitative predictions of cell behavior for a given genotype as consequence of the different molecular interactions. Automatic design techniques aim at solving the inverse problem of finding the sequences of nucleotides that better fit a targeted behavior. In the post-genomic era, our molecular knowledge and modeling capabilities have allowed to start using such methodologies with success. Herein, we describe how the emergence of this new type of tools could enable novel synthetic biology applications. We highlight the essential elements to develop automatic design procedures for synthetic biology pointing out their advantages and bottlenecks. We discuss in detail the experimental difficulties to overcome in the in vivo implementation of designed networks. The use of automatic design to engineer biological networks is starting to emerge as a new technique to perform synthetic biology, which should not be neglected in the future

FEBS Letters 586:15, 2037-2042, doi: 10.1016/j.febslet.2012.02.031.

DNA biosensors that reason

Iñaki Sainz de Murieta and Alfonso Rodríguez-Patón,

Despite the many designs of devices operating with the DNA strand displacement, surprisingly none is explicitly devoted to the implementation of logical deductions. The present article introduces a new model of biosensor device that uses nucleic acid strands to encode simple rules such as “IF DNA _ strand1 is present THEN diseaseA” or “IF DNA _ strand1 AND DNA _ strand2 are present THEN diseaseB”. Taking advantage of the strand displacement operation, our model makes these simple rules interact with input signals (either DNA or any type of RNA) to generate an output signal (in the form of nucleotide strands). This output signal represents a diagnosis, which either can be measured using FRET techniques, cascaded as the input of another logical deduction with different rules, or even be a drug that is administered in response to a set of symptoms. The encoding introduces an implicit error cancellation mechanism, which increases the system scalability enabling longer inference cascades with a bounded and controllable signal–noise relation. It also allows the same rule to be used in forward inference or backward inference, providing the option of validly outputting negated propositions (e.g. “diagnosis A excluded”). The models presented in this paper can be used to implement smart logical DNA devices that perform genetic diagnosis *in vitro*.

BioSystems 109:2, 91-104, doi: 10.1016/j.biosystems.2012.02.005.

Computational design of genomic transcriptional networks with adaptation to varying environments

Javier Carrera, Santiago F. Elena and Alfonso Jaramillo

Transcriptional profiling has been widely used as a tool for unveiling the coregulations of genes in response to genetic and environmental perturbations. These coregulations have been used, in a few instances, to infer global transcriptional regulatory models. Here, using the large amount of transcriptomic information available for the bacterium *Escherichia coli*, we seek to understand the design principles determining the regulation of its transcriptome. Combining transcriptomic and signaling data, we develop an evolutionary computational procedure that allows obtaining alternative genomic transcriptional regulatory network (GTRN) that still maintains its adaptability to dynamic environments. We apply our methodology to an *E. coli* GTRN and show that it could be rewired to simpler transcriptional regulatory structures. These rewired GTRNs still maintain the global physiological response to fluctuating environments. Rewired GTRNs contain 73% fewer regulated operons. Genes with similar functions and coordinated patterns of expression across environments are clustered into longer regulated operons. These synthetic GTRNs are more sensitive and show a more robust response to challenging environments. This result illustrates that the natural configuration of *E. coli* GTRN does not necessarily result from selection for robustness to environmental perturbations, but that evolutionary contingencies may have been important as well. We also discuss the limitations of our methodology in the context of the demand theory. Our procedure will be useful as a novel way to analyze global transcription regulation networks and in synthetic biology for the *de novo* design of genomes.

PNAS, August 27 2012, doi:10.1073/pnas.1200030109.

An automated approach for single-cell tracking in epifluorescence microscopy applied to E. coli growth analysis on microfluidics biochips

Catalin Fetita, Boris Kirov, Alfonso Jaramillo and Christophe Lefevre

With the accumulation of knowledge for the intimate molecular mechanisms governing the processes inside the living cells in the later years, the ability to characterize the performance of elementary genetic circuits and parts at the single-cell level is becoming of crucial importance. Biological science is arriving to the point where it can develop hypothesis for the action of each molecule participating in the biochemical reactions and need proper techniques to test those hypothesis. Microfluidics is emerging as the technology that combined with high-magnification microscopy will allow for the long-term single-cell level observation of bacterial physiology. In this study we design, build and characterize the gene dynamics of genetic circuits as one of the basic parts governing programmed cell behavior. We use *E. coli* as model organism and grow it in microfluidics chips, which we observe with epifluorescence microscopy. One of the most invaluable segments of this technology is the consequent image processing, since it allows for the automated analysis of vast amount of single-cell observation and the fast and easy derivation of conclusions based on that data. Specifically, we are interested in promoter activity as function of time. We expect it to be oscillatory and for that we use GFP (green fluorescent protein) as a reporter in our genetic circuits. In this paper, an automated framework for single-cell tracking in phase-contrast microscopy is developed, combining 2D segmentation of cell time frames and graph-based reconstruction of their spatiotemporal evolution with fast tracking of the associated fluorescence signal. The results obtained on the investigated biological database are presented and discussed.

Proc. SPIE 8317, doi: 10.1117/12.911371

Continuous computation in engineered gene circuits

Angel Goñi-Moreno and Martyn Amos

In this paper we consider the problem of representation and measurement in genetic circuits, and investigate how they can affect the reliability of engineered systems. We propose a design scheme, based on the notion of continuous computation, which addresses these issues. We illustrate the methodology by showing how a concept from computer architecture (namely, branch prediction) may be implemented *in vivo*, using a distributed approach. Simulation results confirm the in-principle feasibility of our method, and offer valuable insights into its future laboratory validation

BioSystems 109:1, 52-56, doi: 10.1016/j.biosystems.2012.02.001.

Munich, March 2012
Second annual project review

Fifteen members of the BACTOCOM project consortium met with EU Project Officer, Béatrice Marquez-Garrido, and two reviewers, Professors Eörs Szathmáry and Giuditta Franco, at the end of March for the second periodic review of the project. The meeting was hosted by Professor Fredrich Simmel at the Technische Universität München (TUM) Bionanotechnology Laboratory in Garching, near Munich.

Members of the team gave presentations on the progress of each of the elements of the project. This generated lively discussion about the meaning of the results achieved and the future focus for the work. Professor Simmel gave an outline of the development of the Bionanotechnology Laboratory at TUM followed by a tour of the outstanding facilities, showing some of the exciting equipment available for use on the BACTOCOM project.

At the end of the day the reviewers fed back their conclusions about the project and the progress that has been made. They were very happy with the project, feeling that the consortium works together well and has made valuable progress in the last year. They commented:

"Overall we congratulate the whole consortium for very promising progress. It was a pleasure to be here."

Martyn Amos thanked the reviewers on behalf of the BACTOCOM consortium. He also thanked Professor Simmel and his team for hosting the meeting, and being so welcoming to the BACTOCOM team.

A major part of these meetings is the opportunity to discuss the work with colleagues in different institutions face –to –face and informally so it was important that in addition to the hard work of the review meeting the team were also able to find time to relax in Munich admiring the famous Rathaus-Glockenspiel in the Marienplatz at the heart of Munich and sampling the beer for which Munich is renowned.

Featured researcher
Andrea Meyer

Andrea Meyer is a Ph.D. student in the Biomolecular Systems and Bionanotechnology Laboratory of the Physics Department at the Technische Universität München (TUM). She studied physics at the Ludwig-Maximilians-Universität München (LMU). For her diploma she investigated a quorum sensing system in the bacterium *Pseudomonas putida* using single cell fluorescence microscopy.

In her current research, she is working on genetic networks which are cloned and implemented in the bacterium *Escherichia coli*. These systems respond to different external signals and will be connected to logic networks. The read-out is by single cell fluroescence microscopy where the gene expression can be observed by the production of a green fluorescent protein. In time-lapse mode the kinetics and evolution of the systems can be investigated and analyzed.

In addition to her own work in the lab, Andrea was the instructor for the TUM iGEM teams in 2010 and 2011. She led her teams to gold medals in 2010 (bioLOGICS: Logical RNA devices enabling BioBrick-Network formation) and in 2011 (EXPRESS3D: A optogenetical bacterial 3D printer) and to the first world finals at MIT in 2011.

Posters:

Single cell studies of bacterial communication systems. Cas Conference, Munich, 2012

Papers:

A. Meyer, J. Megerle, C. Kuttler, J. Müller, C. Aguilar, L. Eberl, B. Hense, J.O. Rädler. Dynamics of AHL mediated quorum sensing under flow and non-flow conditions. *Physical Biology*, 9 (2012) 026007

In this section we profile a member of our External Advisory Board, or an external collaborator.

Advisory board
Jeff Hasty

Prof. Hasty is a Research Professor in Bioengineering at the University of California San Diego (UCSD) Jacobs School of Engineering in the USA. His research interests are computational genomics and the dynamics of gene regulatory networks which control cell function.

Prior to joining UCSD in 2002, Jeff Hasty was an Assistant Research Professor in the Biomedical Engineering Department at Boston University. He received a Ph.D. in physics from Georgia Tech in 1997, and went on to serve as a lecturer at Georgia Tech and post-doctoral fellow at the Supercomputing Research Institute at Florida State University before joining Boston University. A promising biophysicist/mathematical biologist, Hasty's research on gene networks is supported by DARPA, NSF, and the Fetzer Institute.

Hasty's long-term goal is to build synthetic genetic switches or oscillators which could be inserted into a patient's cells to tightly regulate the expression of a desired protein, or even to cause an undesirable cell to self-destruct. Hasty has been an invited speaker on gene regulatory networks at more than 20 professional meetings, and his work has been covered in the popular press including CNN and Business Week.

http://www.jacobsschool.ucsd.edu/faculty/faculty_bios/index.sfe?fmp_recid=187

Project activities

In May, Niall Murphy, postdoctoral researcher at the Universidad Politécnica de Madrid, spent a week with the Intergenomics Group at the University of Cantabria in Santander.

In May, Joao B. Xavier from the Memorial Sloan-Kettering Cancer Center in New York gave three seminars in the Universidad Politécnica de Madrid – Department of Artificial Intelligence Masters program at the invitation of Alfonso Rodriguez-Paton.

In May, Kajetan Bentele a PhD student in the Institute for Theoretical Biology, Humboldt Universitaet zu Berlin visited the Department of Molecular Genetics, Weizmann Institute of Science Rehovot, Israel to give a presentation; "Suppression of mRNA structure shapes codon usage at gene start in bacteria".

In June, Martyn Amos from the Centre for Computing and Informatics Research at Manchester Metropolitan University visited the Institute of Theoretical Biology at the Charité - Universitätsmedizin Berlin to give a seminar on molecular computing.

In June, Ángel Goñi Moreno, postdoctoral researcher in the Centre for Computing and Informatics Research at Manchester Metropolitan University, visited the Intergenomics Group at the University of Cantabria to work with them.

At the end of July Ilka Axmann and Tim Kolmsee from the Institute for Theoretical Biology at Charité-Universitätsmedizin Berlin attended the CAS Conference on Synthetic Biology at Ludwig-Maximilians-Universität (LMU) in Munich. It was an interesting meeting, with fruitful discussions and news on advances in the field of synthetic biology. Tim presented a poster about his work on the RNA comparator. Ilka gave a short talk on 'Designing biocomputing devices based on RNA-RNA interactions'.

In this section we highlight project news items

BACTOCOM partner Alfonso Jaramillo of the Institute of Systems and Synthetic Biology at Genopole-Universite d'Evry Val d'Essonne-CNRS was an invited speaker at the conference, giving a talk entitled 'Automatic Design of RNA and Transcriptional Circuits in *E.coli*'.

BACTOCOM was well represented at the conference as Ilka and Tim also met Friedrich Simmel from Technische Universität München there .

Project news

BACTOCOM partner sees nanosystems success

Professor Friedrich Simmel, leader of the Munich BACTOCOM site, has received significant funding to continue the "Nanosystems Initiative Munich - NIM" Cluster of Excellence. This prestigious award will allow NIM to continue for at least another five years within the German Excellence Initiative. The final decisions were made on 15th June, and NIM is one of 43 Centres of Excellence announced. Professor Simmel (Technische Universität München) is co-coordinator of NIM, together with Professor Jochen Feldmann of Ludwig-Maximilians-Universität München.

NIM is one of the world's leading centres for nanoscience, combining the expertise of about 200 scientists from ten institutions in Munich and Augsburg. The field of nanoscience is very broad; within NIM it reaches from information technology to nanoenergy to the life sciences. NIM's main focus is on nanosystems. While many individual nanoscale building blocks and components have been devised in recent years, little is known about their integration into functional systems working in complex environments. Professor Simmel is working with the BACTOCOM project in this area, developing a prototype biochemical computing framework. The overall objective of the Cluster of Excellence Nanosystems Initiative Munich (NIM) in the first funding period was the development, production, and control of artificial and multifunctional nanosystems for information technology, energy conversion and medically relevant technologies. In the future, the integration of these nanosystems into complex and realistic environments will be a central aspect. Within NIM scientists from physics, biophysics, physical chemistry, biochemistry, pharmacology, biology, electrical engineering, and medicine from the Munich area work together. Through its highly successful work in the first period with more than 1000 scientific publications, the NIM cluster has become an internationally recognized centre for nanoscience. The cluster focuses on five fields of competence: quantum-nano physics, nano-hybrid systems, nanosystems for energy conversion, biomolecular nanosystems and biomedical nanosystems. An important strategic goal of the cluster is to attract outstanding young scientists and provide them with optimal working conditions.

Clusters of Excellence are funded by the German Federal and State Governments as part of the Excellence Initiative. Clusters of Excellence enable the establishment of internationally visible, competitive research and training facilities, thereby enhancing scientific networking and cooperation among the participating institutions. They are intended to form an important part of a university's strategic and thematic planning, significantly raise its profile and reflect its considered long-term priorities. In conjunction with the other two elements of the Excellence Initiative; graduate schools and institutional strategies to promote top-level research, Clusters of Excellence are intended to increase Germany's attraction as a research location in the long term and improve its international competitiveness.

For more information on NIM, please see http://www.nano-initiative-munich.de/

Contact: Professor Friedrich Simmel simmel@tum.de

In this section we highlight project news items

BACTOCOMINFO

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Please contact us if you need any further information about the project

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Contact

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Project number 248919.

Project coordinator:
Prof. Martyn Amos
School of Computing,
Mathematics and Digital Technology
Manchester Metropolitan University
Manchester M15GD UK

E: M.Amos@mmu.ac.uk
T: +44 (0)161 247 1534

http://www.bactocom.eu