Bacterial Computing with Engineered Populations

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Abstract

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BACTOCOMNEWS

July 2011

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

www.bactocom.eu

BACTOCOM gains momentum

Coordinator's report, Dr Martyn Amos

I'm delighted to report that the project successfully navigated its first annual review in Brussels.

In April we were privileged to have Professors Eors Szathmary and David Gilbert assess the project's progress, and the outcome of the review was positive.

This was a big milestone for the project, as it marked the first major external validation of our approach. As a result of the discussions, we have slightly modified the work programme, which is perfectly normal for a project of this complexity.

We published five papers since the last newsletter; details of each of these are given inside.

Project members met in Berlin this month for a periodic meeting; see page 4 for a report.

A new section of the newsletter is the "Featured researcher", in which we profile a BACTOCOM participant. This time we talk to Angel Goni-Moreno from MMU

BACTOCOM has contributed to several European initiatives, including the Future and Emerging Technologies Conference and Exhibition, held in Budapest in May, and the new COBRA coordination action (details inside).

Overall, the project is well-placed to build on its achievements so far, and we look forward to the next six months with renewed optimism!

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Optimal viral strategies for bypassing RNA silencing

Guillermo Rodrigo, Javier Carrera, Alfonso Jaramillo and Santiago F. Elena

The RNA silencing pathway constitutes a defence mechanism highly conserved in eukaryotes, especially in plants, where the underlying working principle relies on the repressive action triggered by the intracellular presence of double-stranded RNAs. This immune system performs a post-transcriptional suppression of aberrant mRNAs or viral RNAs by small interfering RNAs (siRNAs) that are directed towards their target in a sequence-specific manner. However, viruses have evolved strategies to escape from silencing surveillance while promoting their own replication. Several viruses encode suppressor proteins that interact with different elements of the RNA silencing pathway and block it. The different suppressors are not phylogenetically nor structurally related and also differ in their mechanism of action. Here, we adopt a model-driven forward-engineering approach to understand the evolution of suppressor proteins and, in particular, why viral suppressors preferentially target some components of the silencing pathway. We analysed three strategies characterized by different design principles: replication in the absence of a suppressor, suppressors targeting the first protein component of the pathway and suppressors targeting the siRNAs. Our results shed light on the question of whether a virus must opt for devoting more time into transcription or into translation and on which would be the optimal step of the silencing pathway to be targeted by suppressors. In addition, we discussed the evolutionary implications of such designing principles.

Journal of the Royal Society Interface **6**:8, 6 February 2011, 257-268. doi: 10.1098/ rsif.2010.0264.

Model for a population-based microbial oscillator

Angel Goni-Moreno and Martyn Amos

Genetic oscillators are a major theme of interest in the emerging field of synthetic biology. Until recently, most work has been carried out using intra-cellular oscillators, but this approach restricts the broader applicability of such systems. Motivated by a desire to develop large-scale, spatially distributed cell-based computational systems, we present an initial design for a population-level oscillator which uses three different bacterial strains. Our system is based on the client–server model familiar to computer science, and uses quorum sensing for communication between nodes. Importantly, it is robust to perturbation and noise. We present the results of extensive in silico simulation tests, which confirm the feasibility of our design.

BioSystems **105**:3, 6 June 2-11, 286-294. doi: 10.1016/j.biosystems. 2011.05.011.

Simulating a rock-scissors-paper bacterial game with a discrete cellular automaton

Pablo Gomez-Esteban and Alfonso Rodriguez-Paton

This paper describes some of the results obtained after the design and implementation of a discrete cellular automata simulating the generation, degradation and diffusion of particles in a two dimensional grid where different colonies of bacteria coexist and interact. This lattice-based simulator use a random walk-based algorithm to diffuse particles in a 2D discrete lattice. As first results, we analyze and show the oscillatory dynamical behavior of 3 colonies of bacteria competing in a non-transitive relationship analogous to a Rock-Scissors-Paper game (Rock bacteria beats Scissors bacteria that beats Paper bacteria; and Paper beats Rock bacteria). The interaction and communication between bacteria is done with the quorum sensing process through the generation and diffusion of three small molecules called autoinducers. These are the first results obtained from the first version of a general simulator able to model some of the complex molecular information processing and rich communication processes in synthetic bacterial ecosystems.

New Challenges on Bioinspired Applications, Lecture Notes in Computer Science, 2011, Volume 6687, 363-370, doi:10.1007/978-3-642- 21326-7_39

Biomolecular computers

Inaki Sainz de Murieta, Jesus Miro-Bueno and Alfonso Rodriguez-Paton

Biomolecular computation and synthetic biology are the main disciplines in the design and implementation of biological computing devices. This article examines some of the key works concerning this type of logical devices processing biological information. Their design and construction follow two main approaches. The first approach builds computing devices based on the properties of nucleic acids, whereas the second approach focuses on genetic regulatory networks. Examples of the nucleic acid based approach are DNA self-assembly, DNA automata based on restriction enzymes or deoxyribozymes and logic circuits based on DNA strand displacement. Examples of the use of genetic networks are NOT, AND and OR logic gates, a genetic toggle switch that works like a biological memory unit, and several genetic oscillators that work as biological clocks.

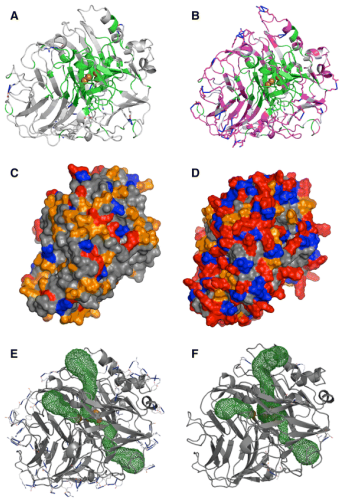
Current Bioinformatics **6**:2, June 2011, 173-183.

Pushing the limits of automatic computational protein design: design, expression, and characterization of a large synthetic protein based on a fungal laccase scaffold

Doris J. Glykys, Geza R. Szilvay, Pablo Tortosa, Maria Suarez Diaz, Alfonso Jaramillo and Scott Banta

The de novo engineering of new proteins will allow the design of complex systems in synthetic biology. But the design of large proteins is very challenging due to the large combinatorial sequence space to be explored and the lack of a suitable selection system to guide the evolution and optimization. One way to approach this challenge is to use computational design methods based on the current crystallographic data and on molecular mechanics. We have used a laccase protein fold as a scaffold to design a new protein sequence that would adopt a 3D conformation in solution similar to a wild-type protein, theTrametes versicolor (TvL) fungal laccase. Laccases are multi-copper oxidases that find utility in a variety of industrial applications. The laccases with highest activity and redox potential are generally secreted fungal glycoproteins. Prokaryotic laccases have been identified with some desirable features, but they often exhibit low redox potentials. The designed sequence (DLac) shares a 50% sequence identity to the original TvL protein. The new DLac gene was overexpressed in E. coli and the majority of the protein was found in inclusion bodies. Both soluble protein and refolded insoluble protein were purified, and their identity was verified by mass spectrometry. Neither protein exhibited the characteristic T1 copper absorbance, neither bound copper by atomic absorption, and neither was active using a variety of laccase substrates over a range of pH values. Circular dichroism spectroscopy studies suggest that the DLac protein adopts a molten globule structure that is similar to the denatured and refolded native fungal TvL protein, which is significantly different from the natively secreted fungal protein. Taken together, these results indicate that the computationally designed DLac expressed in E. coli is unable to utilize the same folding pathway that is used in the expression of the parent TvL protein or the prokaryotic laccases. This sequence can be used going forward to help elucidate the sequence requirements needed for prokaryotic multi-copper oxidase expression.

Systems and Synthetic Biology **5**:1-2, 45-58. doi: 10.1007/s11693-011-9080-9



Berlin, July 2011
Periodic project review meeting

Project members met in Berlin this month for an regular get-together to discuss progress.

Over twenty participants were hosted by Nils Bluethgen and Ilka Axmann of the Charite - Universitätsmedizin site. The Charité is a joint institution of the Freie Universität Berlin and the Humboldt-Universität zu Berlin, and is one of the largest university hospitals in Europe. It has a staff of 3800 doctors and scientists; more than half of the German Nobel Prize winners in medicine and physiology come from the Charité, among them Emil von Behring, Robert Koch and Paul Ehrlich. The Charité also has an international reputation for excellence in training. It extends over four campuses with more than 100 clinics and institutes bundled under 17 CharitéCenters. With more than 12,000 employees, the Charité generates about 1.2 billion euros in sales per year and is one of the largest employers in Berlin. It celebrated its 300-year anniversary in 2010.

In addition to the scientific discussions, we enjoyed a barbeque outside the meeting room, and a visit to the Museum fur Naturkunde (Natural History Museum), featuring the world's tallest dinosaur skeleton!

The meeting was closed by Fernando de la Cruz, who gave a seminar on bacterial conjugation. Our next meeting will be held in Madrid, in January 2012.

Featured researcher
Angel Goni-Moreno

Angel works on computational modelling as part of the Manchester Metropolitan University team.

He was born in Madrid in 1983, and began his studies in 2001, in computer engineering at the Universidad Politecnica de Madrid. After earning his degree, he joined the Natural Computing Group at UPM, where he began working on DNA computing and computational biology.

In 2010 he was awarded his European Ph.D. in computer engineering (in the field of bacterial computing). On the way, he collected an M.Sc. in artificial intelligence.

His Ph.D. work used bacterial communities to autonomously solve complex problems, using communication schemes such as conjugation and quorum sensing.

"I like working on BACTOCOM because it helps develop the full potential of my research capabilities in a scientific field for which I have real passion."

Angel Goni-Moreno

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

Advisory board
Natalio Krasnogor

Prof. Krasnogor is a Professor of Applied Interdisciplinary Computing in the School of Computer Science at the University of Nottingham, UK.

He currently holds a prestigious EPSRC Leadership Fellowship in Synthetic Biology, and is a world leader in the field of nature-inspired (and nature-*based*) computation.

He is currently working on an ambitious project to develop a cell-based operating system: as he explains to The Register, "Currently, each time we need a cell that will perform a certain new function we have to recreate it from scratch which is a long and laborious process. Most people think all we have to do to modify behaviour is to modify a cell's DNA but it's not as simple as that — we usually find we get the wrong behaviour and then we are back to square one. If we succeed with this, in five years time we will be programming bacterial cells in the computer and compiling and storing its program into these new cells so they can readily execute them."

http://www.cs.nott.ac.uk/~nxk/

Project activities

MMU is leading a new FP7 coordination action, titled COBRA, and supported by the Future and Emerging Technologies Proactive intitiative.

The project acronym stands for Coordination of Biological and Chemical IT Research Activities, and its main objective is to act as a unifying focus for bio/chem IT, across a range of different research topics.

The project is firmly rooted in the four small and medium scale projects funded under the 7th Framework Programme, BACTOCOM, ECCell, MATCH-IT and NEU-NEU. Considered as a whole, these projects capture a significant proportion of European activity in bio/chem IT. This project will link these existing communities into a "network of networks", in order to promote cohesion, improve the quality and impact of European research, and foster collaborative links with similar activities in other regions (eg. US, Japan). It will achieve this by capturing the current state-of-the-art in European research in this area, organizing small to medium-scale workshops, developing a strategic roadmap document, and working with international partners.

The project was launched in January 2011, and will hold a workshop at the European Conference on Artificial Life, in Paris in August.

http://www.cobra-project.eu

BACTOCOM features in a new exhibition at the Manchester Museum of Science and Industry.

Revolution Manchester showcases scientific and technological advances that were either developed or nurtured in Manchester, and BACTOCOM was selected to represent "2013 and beyond".

The exhibit features a panel describing the project, and a video interview with project coordinator Dr Martyn Amos.

In this section we highlight project involvement in external events or initiatives.

BACTOCOMINFO

July 2011

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

Contact

BACTOCOM is supported by the European Commission, funded under the Seventh Framework Programme: Future and Emerging Technologies (FET) Proactive: Bio-chemistry-based Information Technology (CHEM-IT).

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BACTOCOMNEWS

January 2012

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

www.bactocom.eu

High-impact papers

Coordinator's report, Dr Martyn Amos

This newsletter brings news of several new high-impact outputs from the project, including papers in *PNAS* and *PLoS ONE*. We're also pleased to announce our first Master's thesis!

Project members met this month in Madrid for a periodic meeting; see page 4 for a report .

In the profile section we introduce Ph.D. student Thomas Landrain, and highlight his attempts to democratise biology. We also discuss a related DIYbio project we're running in Manchester.

Our next annual review is looming large, so the June newsletter will give an update on how the project has responded to that.

The deadline for the next round of FP7 funding passed this month - as one would expect, project participants are involved in several proposals, so we will hope to hear news in a few month's time.

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Timing molecular motion and production with a synthetic transcriptional clock

Elisa Franco, Eike Friedrichs, Jongmin Kim, Ralf Jungmann, Richard Murray, Erik Winfree and Friedrich Simmel

The realization of artificial biochemical reaction networks with unique functionality is one of the main challenges for the development of synthetic biology. Due to the reduced number of components, biochemical circuits constructed in vitro promise to be more amenable to systematic design and quantitative assessment than circuits embedded within living organisms. To make good on that promise, effective methods for composing subsystems into larger systems are needed. Here we used an artificial biochemical oscillator based on in vitro transcription and RNA degradation reactions to drive a variety of "load" processes such as the operation of a DNA-based nanomechanical device ("DNA tweezers") or the production of a functional RNA molecule (an aptamer for malachite green). We implemented several mechanisms for coupling the load processes to the oscillator circuit and compared them based on how much the load affected the frequency and amplitude of the core oscillator, and how much of the load was effectively driven. Based on heuristic insights and computational modeling, an "insulator circuit" was developed, which strongly reduced the detrimental influence of the load on the oscillator circuit. Understanding how to design effective insulation between biochemical subsystems will be critical for the synthesis of larger and more complex systems.

Proceedings of the National Academy of Sciences of the USA **108**:40, 4 October 2011, E784-E793. doi: 10.1073/pnas.1100060108.

A simple negative interaction in the positive transcriptional feedback of a single gene is sufficient to produce reliable oscillations

Jesús M. Miró-Bueno and Alfonso Rodríguez-Patón

Negative and positive transcriptional feedback loops are present in natural and synthetic genetic oscillators. A single gene with negative transcriptional feedback needs a time delay and sufficiently strong nonlinearity in the transmission of the feedback signal in order to produce biochemical rhythms. A single gene with only positive transcriptional feedback does not produce oscillations. Here, we demonstrate that this single-gene network in conjunction with a simple negative interaction can also easily produce rhythms. We examine a model comprised of two well-differentiated parts. The first is a positive feedback created by a protein that binds to the promoter of its own gene and activates the transcription. The second is a negative interaction in which a repressor molecule prevents this protein from binding to its promoter. A stochastic study shows that the system is robust to noise. A deterministic study identifies that the dynamics of the oscillator are mainly driven by two types of biomolecules: the protein, and the complex formed by the repressor and this protein. The main conclusion of this paper is that a simple and usual negative interaction, such as degradation, sequestration or inhibition, acting on the positive transcriptional feedback of a single gene is a sufficient condition to produce reliable oscillations. One gene is enough and the positive transcriptional feedback signal does not need to activate a second repressor gene. This means that at the genetic level an explicit negative feedback loop is not necessary. The model needs neither cooperative binding reactions nor the formation of protein multimers.

Therefore, our findings could help to clarify the design principles of cellular clocks and constitute a new efficient tool for engineering synthetic genetic oscillators.

PLoS ONE **6**:11, 10 November 2011, e27414. doi: 10.1371/journal.pone.0027414.

Computational design of synthetic regulatory networks from a genetic library to characterize the designability of dynamical behaviors

Guillermo Rodrigo, Javier Carrera and Alfonso Jaramillo

The engineering of synthetic gene networks has mostly relied on the assembly of few characterized regulatory elements using rational design principles. It is of outmost importance to analyze the scalability and limits of such a design workflow. To analyze the design capabilities of libraries of regulatory elements, we have developed the first automated design approach that combines such elements to search the genotype space associated to a given phenotypic behavior. Herein, we calculated the designability of dynamical functions obtained from circuits assembled with a given genetic library. By designing circuits working as amplitude filters, pulse counters and oscillators, we could infer new mechanisms for such behaviors. We also highlighted the hierarchical design and the optimization of the interface between devices. We dissected the functional diversity of a constrained library and we found that even such libraries can provide a rich variety of behaviors. We also found that intrinsic noise slightly reduces the designability of digital circuits, but it increases the designability of oscillators. Finally, we analyzed the robust design as a strategy to counteract the evolvability and noise in gene expression of the engineered circuits within a cellular background, obtaining mechanisms for robustness through non-linear negative feedback loops.

Nucleic Acids Research **39**(20), 24 August 2011, e138. doi: 10.1093/nar/gkr616.

Biological and chemical information technologies

Martyn Amos, Peter Dittrich, John McCaskill and Steen Rasmussen

Biological and chemical information technologies (bio/chem IT) have the potential to reshape the scientific and technological landscape. In this paper we briefly review the main challenges and opportunities in the field, before presenting several case studies based on ongoing FP7 research projects.

Procedia Computer Science **7**, 56-60. doi: 10.1016/j.procs.2011.12.019

Empirical model and in vivo characterization of the bacterial response to synthetic gene expression show that ribosome allocation limits growth rate

Javier Carrera, Guillermo Rodrigo, Vijai Singh, Boris Kirov and Alfonso Jaramillo

Synthetic biology uses modeling to facilitate the design of new genetic constructions. In particular, it is of utmost importance to model the reaction of the cellular chassis when expressing heterologous systems. We constructed a mathematical model for the response of a bacterial cell chassis under heterologous expression. For this, we relied on previous characterization of the growth-rate dependence on cellular resource availability (in this case, DNA and RNA polymerases and ribosomes). Accordingly, we estimated the maximum capacities of the cell for heterologous expression to be 46% of the total RNA and the 33% of the total protein. To experimentally validate our model, we engineered two genetic constructions that involved the constitutive expression of a fluorescent reporter in a vector with a tunable origin of replication. We performed fluorescent measurements using population and single-cell fluorescent measurements. Our model predicted cell growth for several heterologous constructions under five different culture conditions and various plasmid copy numbers with significant accuracy, and confirmed that ribosomes act as the limiting resource. Our study also confirmed that the bacterial response to synthetic gene expression could be understood in terms of the requirement for cellular resources and could be predicted from relevant cellular parameters.

Biotechnology Journal **6**(7), July 2011, 773-783. doi:10.1002/biot.201100084.

Autonomous resolution based on DNA strand displacement

Alfonso Rodriguez-Paron, Inaki Sainz de Murieta and Petr Sosik

We present a computing model based on the technique of DNA strand displacement which performs a chain of logical resolutions with logical formulae in conjunctive normal form. The model is enzyme-free and autonomous. Each clause of a formula is encoded in a separate DNA molecule: propositions are encoded assigning a strand to each proposition p, and its complementary strand to the proposition ¬p; clauses are encoded comprising different propositions in the same strand. The model allows to run logic programs composed of Horn clauses by cascading resolution steps and, therefore, possibly function as an autonomous programmable nano-device. This technique can be also used to solve SAT. The resulting SAT algorithm has a linear time complexity in the number of resolution steps, whereas its spatial complexity is exponential in the number of variables of the formula.

DNA Computing and Molecular Programming (Proc. DNA17), Lecture Notes in Computer Science 6937, 2011, 190-203. doi: 10.1007/978-3-642-23638-9_16

Genetically engineered light sensors for control of bacterial gene expression

Daniel Camsund, Peter Lindblad and Alfonso Jaramillo

Light of different wavelengths can serve as a transient, noninvasive means of regulating gene expression for biotechnological purposes. Implementation of advanced gene regulatory circuits will require orthogonal transcriptional systems that can be simultaneously controlled and that can produce several different control states. Fully genetically encoded light sensors take advantage of the favorable characteristics of light, do not need the supplementation of any chemical inducers or co-factors, and have been demonstrated to control gene expression in *Escherichia coli*. Herein, we review engineered light-sensor systems with potential for in vivo regulation of gene expression in bacteria, and highlight different means of extending the range of available light input and transcriptional output signals. Furthermore, we discuss advances in multiplexing different light sensors for achieving multichromatic control of gene expression and indicate developments that could facilitate the construction of efficient systems for light-regulated, multistate control of gene expression.

Biotechnology Journal **6**(7), July 2011, 826-836. doi:10.1002/biot.201100091.

A first approach to individual-based modeling of the bacterial conjugation dynamics

Antonio Prestes Garcia

This work presents a spatially explicit individual-based model of bacterial conjugation using use a discrete representation of time and space. The space is defined by a discrete grid where agents are place and evolve through their local interactions. Each agent is described by a state vector updated according the model rules which takes into account the local agent state and the states of close neighbor agents located at adjacent cells. The rules for updating the states of each individual represent metabolic processes such as nutrient uptake, growth, reproduction and conjugation of each bacterial cell in the colony. It also takes into account also non-metabolic processes such as nutrient diffusion.

M.Sc. thesis, Computer Faculty, Universidad Politecnica de Madrid. http://oa.upm.es/10478/

Madrid, January 2012
Periodic project review meeting

Project members met in Madrid this month for an regular get-together to discuss progress.

Participants were hosted by Alfonso Rodriguez-Paton of the Universidad Politecnica de Madrid (UPM) site. The Technical University of Madrid celebrated its 25th anniversary in 1996, although the majority of its centres are over hundreds of years old and were founded in the 18th and 19th centuries. Each of them maintained its independence until being grouped together to form the UPM. It is no exaggeration to state that over one and a half centuries, a great part of the history of Spanish technology has been written by the Schools of Architecture and Engineering.

In addition to the scientific discussions, we enjoyed dinner at the famous (and eccentric!) Gabino Teca restaurant.

Featured researcher
Thomas Landrain

Thomas is a Ph.D. student in synthetic biology at the Institute of Systems and Synthetic Biology.

After studying molecular biology and physiology, he gained Masters degrees in genetics and interdisciplinary life sciences.

He is also the president and co-founder of La Paillassse, a "physical and web platform for citizen scientists, amateur biologists, researchers and entrepreneurs that fosters open-science, debates and hands-on practice of Biotechnologies."

He was a finalist in the 2007 iGEM competition with the Paris team.

"I'm more and more interested in the systems biology approach of life, and more particularly in the possibilities that synthetic biology offers to humans for the future."

Thomas Landrain

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

Advisory board
Jane Calvert

Dr Calvert is an RCUK (Research Councils UK) Academic Fellow at Innogen, University of Edinburgh, UK.

Jane has an undergraduate degree in Human Sciences from Sussex and an MSc in the History and Philosophy of Science from the LSE. She did her doctoral work at SPRU, University of Sussex on the idea of 'basic research'. She worked as a research fellow at SPRU, and then at the ESRC Centre for Genomics in Society (Egenis) at the University of Exeter.

Her broad area of research interest is in the sociology of the life sciences. She is currently studying the emergence, development and epistemic aspirations of the new fields of systems biology and synthetic biology.

http://www.sps.ed.ac.uk/staff/innogen/calvert_jane

Project activities

MMU academics are involved with an innovative new approach to engaging the public with biology, and with science in general.

Manchester DIYbio is a new collaborative project, based at the Manchester Digital Laboratory (MadLab), and funded by the Wellcome Trust.

The 15 month project (which started in March 2011) has created an innovative "citizen science" community, to enable wider participation in biological research. Amateur scientists collaborate with researchers from MMU to develop and carry out a wide range of experiments, as part of the growing worldwide 'biohacking' community.

Successful projects so far include the Manchester Microbe Map (a bacterial "atlas" of the city centre), workshops on topics as diverse as octopus dissection and microbial fuel cells, the first ever UK DIYbio summit meeting, and the construction of a "homebrew" thermal cycler, which we intend to run against both our brand new OpenPCR machine and a commercial machine, in what we are calling the "PCR Challenge".

In addition to creating and encouraging a whole new set of citizen scientists in Manchester and beyond, the project has successfully drawn positive attention to the emerging DIY biology movement.

This has manifested itself in numerous press appearances, radio interviews and even filming with the BBC's main Science Editor, David Shukman (below)

DIYBIO_MCR

http://diybio.madlab.org.uk

In this section we highlight project involvement in external events or initiatives.

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

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Contact

BACTOCOM is supported by the European Commission, funded under the Seventh Framework Programme: Future and Emerging Technologies (FET) Proactive: Bio-chemistry-based Information Technology (CHEM-IT).

Project number 248919.

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