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Synthetic Biology is rapidly consolidating as one of the important fields with significant potential applications and implications in biotechnology. It poses a question on how the scientific and the engineering research might be best coordinated in order to understand, refine, measure, and standardise biomolecules and systems in support of their broad application. Practitioners in this arena have identified the issue of standardization as one of the pillars of the field if the synthetic biology is to become an authentic engineering discipline.

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EUROPEAN COMMISSION

«Towards Standards in Synthetic Biology»

An Exploratory Workshop of the US-EU Task Force in Biotechnology

Segovia, SPAIN
04-06 June 2010

Summary Record

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Directorate-General for Research and Innovation
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FOREWORD

Since 1990, the **EU-US Task Force on Biotechnology Research** has been coordinating transatlantic efforts to guide and exploit the ongoing revolution in biotechnology and the life sciences. The Task Force was established in June 1990 by the European Commission and the US Office of Science and Technology Policy and has since then acted as an effective forum for discussion, coordination and development of new ideas.

The workshop of the *EU-US Task Force on Biotechnology Research* on "**Standards in Synthetic Biology**" was organised on 4-6 June 2010 in the Parador de Segovia (Spain) and hosted by the Centro Nacional de Biotecnología, of the Consejo Superior de Investigaciones Cientificas. Like all the activities of the Task Force, it was designed to create synergies and enhance collaboration between leading EU and US scientists in a cutting-edge field of research. The Directorate General for Research of the European Commission and the US National Science Foundation provided the Administrative and financial support. Professors Victor de Lorenzo (Centro Nacional de Biotecnología, CSIC, Madrid, ES) and Drew Endy (Stanford University, USA) were the Convenors of this scientific event.

The Workshop in Segovia was organised as a high-level EU-US discussion on how scientific and engineering research might be best coordinated in order to understand, refine, measure, and, as possible, standardise biomolecules and systems in support of their broad application. From one perspective, new tools such as *de novo* genome construction are challenging synthetic biologists to become 100s of times better at reliably programming the functional molecular elements that comprise cells. From another perspective, the complexity of biology continues to challenge systems biologists to develop physical representations of cellular behaviour that transcend the simple recapitulation of past observations. Therefore, the core of the Workshop was how both sides of the Atlantic can best work together to increase the capacities for understanding and engineering biological systems at the genome scale.

Twenty-four internationally-renowned senior scientists from EU and the USA were invited to contribute to the theme of "Standards in Synthetic Biology". Observers, also, related to the Task Force were present. The outcome of the Segovia Workshop demonstrated the need of the creation of a Working Group within the Task Force devoted to Synthetic Biology. This Working Group will focus on fostering interaction between scientists working on issues relevant to Synthetic Biology in EU and the USA.

We would like to express our gratitude to professors Drew Endy and Victor de Lorenzo for their outstanding efforts in conveying the meeting and for their contribution to this report,. The coordinators of this activity were Dr Ioannis Economidis (European Commission) and Dr Sohi Rastegar (US National Science foundation).

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Co-Convenors:

Drew Endy (Stanford, US)
Víctor de Lorenzo (Centro Nacional de Biotecnología, Spain)

SUMMARY RECORD

The best thing about standards is that there are so many to choose from!

Multiple sources

PREFACE

Biotechnology Task Force and Synthetic Biology Working Group

Established 20 years ago the EU-US Task Force on Biotechnology research aims to promote the information exchange and the coordination in biotechnology research between the programmes funded by the European Commission and the US Government funding agencies (USDA, DOE, NIH, NSF and NOAA). Over the years the task force has become a successful think tank on Biotechnology Research with a strong forward looking approach and with concrete examples of successful coordination in the areas of: environmental biotechnology, plant biotechnology, biobased products, marine genomics etc. The need for this transatlantic collaboration within this unique forum will certainly be maintained, and is expected to be even deepened, in the near future.

Synthetic Biology is rapidly consolidating as one of the important fields with significant potential applications and implications in biotechnology. There have been a number of efforts throughout the world to identify the barriers and realize the potential of the Synthetic Biology. The sunthetic biology expertise in US and in EU could be considered both - complementary and overlapping. Thus there was a need to allow sharing and cooperation across national and regional boundaries in order to make coherent and efficient progress in the area of synthetic biology. Just like with all new and emerging technologies, there are some unknowns with respect to the environmental and health impacts of synthetic biology. Given the potential of the synthetic biology to change the way we do molecular biology or metabolic engineering, there was a compelling need for a common EU-US work sharing the same views on safety, biosecurity, ethics and education issues. As a logical consequence a Synthetic Biology Working Group under the EU-US Task Force on Biotechnology was established in 2010. This Working Group aims on the fostering of the exchange of views and collaboration in scientific and technical implementation of synthetic biology principles in such areas as standards, orthogonality, minimal genomes, ethics, biosafety (including environmental safety), biosecurity, and education.

Over the next 5 years the working group will focus on standardisation needs, which were not met or realized yet via current practice. It will follow ethical, legal and social issues in relationship to the scientific and technical progress of synthetic biology; and will pay

special attention to the contribution of synthetic biology to the different domains of Biotechnology.

In the light of this background and the stated needs the Working Group organized a successful workshop in Segovia in June 2010. In this workshop it was agreed that as initial direction the working group should be looking to standards to allow communication and material exchange, and technique development.

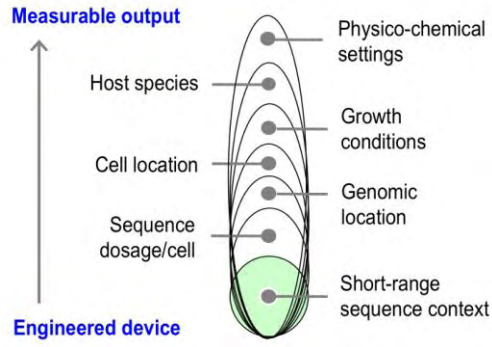
WHY STANDARDS FOR ENGINEERING BIOSYSTEMS AND WHY NOW

Synthetic Biology refers to [1] the design and fabrication of biological components and systems that do not already exist in the natural world and/or [2] the re-design and refactoring of existing biological systems. For decades, scientists have been routinely deleting or inserting genes into microbes, mice, and food crops, to name but a few, in the course of devising new therapeutics, improved foods, and healthier products. A recent addition of attitudes and methodological approaches from computer sciences and mechanical engineering are now bringing benefits. They are greatly strengthening synthetic biology capabilities for achieving complex genetic assemblies, which previously were considered too difficult or lengthy to pursue. Practitioners in this arena have identified the issue of standardization as one of the pillars of the field if SB is to become an authentic engineering discipline. Only by adopting standards on physical and functional formats, assembly methods, measurements and descriptive languages (including biological counterparts to computer *operative systems*) a large international community of SB practitioners can join forces to make possible what could be otherwise considered unfeasible. This is because one can breakdown and develop at various times and places the different stages involved in any synthetic setup: blueprint of the design, synthesis and production of the constituents, assembly of the components and eventual deployment. This is the grand takehome lesson of modern engineering and computer science that Synthetic Biology is now ripe to take.

The basic notion behind SB is that any biological system can be considered as a complex combination of functional, stand-alone elements not unlike those found in man-made devices, and can thus be-deconstructed in a limited number of components and reconstructed in an entirely different configuration for the sake of modifying existing properties or creating altogether new ones. In this context, *Engineering* as a discipline transits from being an *analogy* of the rational combination of genes made possible by modern Molecular Biology and Biotechnology to being a veritable *methodology* to construct complex systems and novel properties based on biological components. SB has an aspect of developing general-use technological and conceptual tools (biological parts, minimal genomes, artificial cells, DNA synthesis), addressing hitherto intractable problems (Biosynthesis of complex molecules, breakdown or recycling of toxic chemicals, biological detection of explosives, biological production of H2 and other fuels) and raising utterly novel challenges (DNA computing, design of biological pattern development, targeting bacteria to tumor cells, expanding the genetic code to non-natural amino acids). Note that

SB is not about *understanding* Biological Systems but about *capitalizing* such systems as a source of components for creating new devices and properties to solve a variety of problems. In that respect, SB maps altogether in the realm of Technology and thus clearly dissociates itself from basic Science –more interested to know and understand how existing Biological Systems work as they are.

One recurrent premise in SB is the vital need to standardize biological components and their interfaces, in a fashion detached from its natural circumstances. Context-independent behaviour of components is clearly a pre-requisite for the robust engineering new devices and properties. While the need and the opportunity of such a formatting have been clearly identified, the success of the endeavor has been quite limited so far. Despite the long list of *biological parts* entered in the open registry kindly supported by the MIT (http://parts.mit.edu), there is still a long way to fulfill the requirements that would raise them to a standard barely comparable to e. g. transistors in electronic circuitry. This is in part due to the intrinsic qualities of biological functions to co-evolve as wholes (and thus behave in a very context-dependent manner), and as a result cannot be described in an easy manner. There is thus a need to develop more robust concepts and a dedicated language to deal and categorize such biological parts, which is based not only in their possible similarity to electronic counterparts, but also on a better comprehension of minimal biological functions -mostly related to regulation of gene expression. These refinements can lay the basis of a future international agreement on the formatting of such parts, their availability and the registry of their users.



Biological context of designed genetic devices. The figure sketches how functional display of a given construct has to go through various layers of biological and physicochemical context before the output can be measured by the observer.

Overcoming the problem of *biological context*

Research in molecular biology of the last 20 years has yielded a wealth of information on the basic features of the gene expression flow. The main instructions encoded in the genome of prokaryotes such as *E. coli* for expression of a given gene or operon rely on 4 types of adjacent DNA sequences: a promoter, an untranslated 5' region (5' UTR) which determines inter alia the binding of ribosomal machinery to the coding sequence (ORF) and a 3' UTR that settles transcription termination and stability. The mRNA sequence can be itself punctuated by 3D motifs and secondary structures that rule its stability or its availability for translation. Many of such expression-related sequences have been studied in detail in their specific context, but the question for us is whether they can be excised from such native cellular milieu, combined with other functional parts and still expect them to behave as before. Traditional genetic engineering has revealed over the years that in many cases such functionalities are maintained in man-made expression devices, but almost never with the same kinetic parameters and quite often displaying outlying behaviours. This poses in all its magnitude the problem of context-dependency of engineered biological functions. As shown in the Figure above the

performance of every expression device in a cell is subject to at least 7 contextual layers ranging from the immediate mutual influence of adjacent DNA sequences all the way to environmental physicochemical conditions. It is essential streamline such itinerary from physical composition to eventual function (i.e. measurable output) by focusing on the earlier layers, in particular in the short-range effects of adjacent sequences. This demands 3 convergent approaches. First, the detailed ***modelling, measurement and parameterization*** of large collections of functional parts in a variety of DNA contexts and growth conditions. Second, the investigation of a ***limited number of archetypal promoters and UTRs*** in various genomic and cellular backgrounds aimed at identifying and ultimately eliminating context-dependency determinants. And third, the forward design of ***orthogonal expression devices*** based on alternative sigma factors and exploitation of parts recruited from mobile genetic elements. The subject of *orthogonal ribosomes* and *alternative genetic codes* are areas of fascinating research but still too exploratory to be the subject of a durable standardization effort.

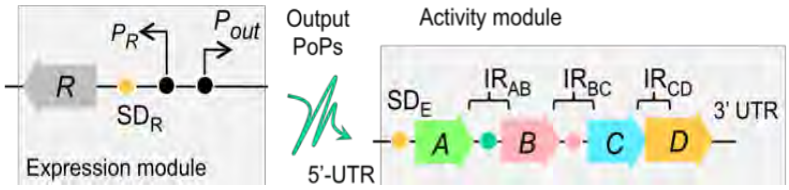
What can and what cannot be standardized

In the world of engineering, the terms *standard* and *standardization* refer mostly to **[a]** the adoption of specific geometric shapes and size formats for the physical assembly of the components of a man-made system (e.g. the sizes and shapes of screw turns), **[b]** the definition of units of measurement of relevant properties and parameters as well as conditions and procedures to calculate them (e.g. Amperes for current, Ohms for resistance, etc), and **[c]** implementation of unambiguous protocols for the manufacture of the engineered objects. These standards allow the abstraction of the properties of the components of a system, their precise description with a suitable -also standardized- quantitative language and the composition of the blueprint of the designed object with identical representation methods. A big bonus in this respect is the possibility to disengage the detailed design of a product from the fabrication of its components and the final assembly of the artefact. This is commonplace in industrial and electronic engineering, but how much of this can be imported into the biological realm?

Apart from the somewhat minor formatting imposed by the nature of the restriction sites used for DNA cloning, Molecular Biology and Biotechnology have been afflicted from their birth by a nearly complete disregard for the standardization issue. There exists a generally chaotic nomenclature for genes and molecular tools, and even the measure of very basic biological functions (e.g. promoter strength) has avoided empirical quantification. It is thus important to examine which of these functions can be the subject of a sound standardization effort which might even derive into a pre-normative background for future international consensus. But what can be standardized with the level of knowledge that we have at this time?

The starting point is the ***establishment of rules for the physical composition of biological (expression) devices.*** As sketched in the adjacent figure below, this involves suitable restriction sites for the ordered addition of a regulatory gene R along with its own

expression signals (promoter P_R and Shine-Delgarno sequence, SD), a target promoter (P_{out}) and an activity module (encoding by default a reporter product) which is endowed with upstream and downstream UTR sequences and containing genes (A,B,C) separated, where necessary, by intergenic regions (IRs). Each of these functional sequences can be punctuated by sites that facilitate the assembly of many device variants, their cloning in vectors tailored to the same end then transfer to a standard bacterial host (see below) and the input / output functions determined in a high-throughput fashion. These devices can then be moved from the assembly / measurement-plasmids into deployment vectors for either stable chromosomal implantation or for plasmid-encoded maintenance. The DNA composition standard will thus enable an easy assembly of individual parts and devices into systems.



A second type of standard deals with the **adoption of RNA polymerase per second (PoPs) units as a universal reference to express promoter strength.** This means on one hand to understand what is, mechanistically, one of these units and to establish experimental benchmarks for its measurement. In this context, it is essential to examine the problem of both intrinsic and extrinsic stochastic variations in transcription that leads to cell-to-cell disparities and ways of projecting such stochastic phenomena into population behaviour. From a more practical side, **conversion tables** between PoPs and some of the most popular reporters (e.g. GFP, LacZ, Lux) could be set up. Furthermore, specific genomic sites that are optimal for directing transcriptional activity of the device into specialized cytoplasmic locations (i.e. *transcription factories,* see below) should be explored. Such sites could also be examined as candidates for standardizing the implantation of the genetic constructs into preset locations of the genomic chassis of *E. coli* and other microorganisms.

Unlike standards of physical assembly and PoPs, establishing comparable complete formats for mRNA and translation is less reachable as an immediate objective. One provisional approach can be the adherence to the same formats for physical assembly as above along with adoption of **benchmarked procedures for examining the influence of mRNA sequence motifs** on final output of the device. Ultimately, computer-assisted design (CAD) should be able to anticipate most possible scenarios of mRNA fate. Since the final level of expression of a given gene product is the result of its transcription, mRNA decay and translation, the correlation of promoter strengths (PoPs) vs. mRNA structures vs. product levels and the generation of rules for their physical drawing and CAD composition will be one of the mosr important challenges to the Synthetic Biology community.

Translation is in itself the other key biological function that checks expression levels of any gene product and its efficiency can also be measured, modelled and parameterized to produce a count of RiPs (*ribosomes per second***)** on a given mRNA. However, for a given

growth condition, RiPs is mostly dependent on the sequence of the target transcript and therefore the relevant parameters can be associated to the mRNA sequence. In any case, as the number of ribosomes depends on growth phase, it might be possible to calculate a **translation efficacy index** deduced from measuring the input/output function of the devices under the various growth and metabolic conditions.

BACKGROUND: STANDARDS FOR ENGINEERING CELL FACTORIES

Scientific Roadmap and Biotechnological needs

Synthetic biology is becoming an inclusive theoretical and applied umbrella to approach biological entities with the conceptual tools and language imported from computational science, electrical circuitry and mechanical manufacturing. The fundamental idea behind synthetic biology is that any biological system can be regarded as a combination of individual functional elements -not unlike those found in man-made devices. These can thus be accurately described as a whole composed of a list of parts that can be combined in novel configurations to modify existing properties or to create new ones. In this context, engineering moves from being an **analogy** of the rational combination of genes -as in standard molecular biology and biotechnology- to becoming a veritable **methodology** with which to construct complex biological systems from first principles. It is a widespread perception that merging authentic (not metaphoric) engineering and molecular biology will certainly have far-reaching consequences.

One central aspect of synthetic biology, which is bound to have an immense impact in Biotechnology, is the possibility of re-programming or creating new live organisms through the logical combination of standardized biological components that are otherwise decoupled from their natural context. In fact, the reliable formatting of biological functionalities and the detailed description of the most basic biological constituents and their interfaces, similar to modern electronic circuits, is one of the challenges of the field. This need is particularly acute in the field of metabolic engineering for synthesis and/or degradation of target chemical molecules, biomaterials and biofuels. Furthermore, standardization of biological functions as predictable inputs/outputs is by no means trivial. There are at least two 2 issues at stake: one is to use Systems Biology to understand the design and operation rules. The second is to exploit such rules for re-engineer biological systems *á la carte*. The challenge is thus to set reliable SOPs, principles and actual units of measurement that are useful for biological engineering, even if we may still miss some fundamental facts.

The EU-US Workshop in Biological Standards

A *Workshop on Standards in Synthetic Biology* was organised on 4-6 June 2010 in Segovia (Spain) as an initiative of the *EU-US Task Force on Biotechnology Research*. The workshop was agreed and endorsed by the Plenary Annual Meeting of the Task Force, last June in Lawrence Berkeley National Laboratory. The purpose of this meeting was to have an informed debate on the setting of standards for formatting various types of biological functions, aimed at engineering robust and predictable cell factories for Biotechnological purposes.

As in former activities of the Task Force, the workshop was designed to create synergies and enhance collaboration between leading EU and US scientists in a cutting-edge field of research (http://ec.europa.eu/research/biotechnology/ec-us/index_en.html)

The Workshop was organized as a high-level EU-US discussion on how scientific and engineering research might be best coordinated in order to understand, refine, measure, and, as possible, standardise biomolecules and systems in support of their broad application. From one perspective, new tools such as *de novo* genome construction are challenging synthetic biologists to become 100s of times better at reliably programming the functional molecular elements that comprise cells. From another perspective, the complexity of biology continues to challenge systems biologists to develop physical representations of cellular behavior that transcend the simple recapitulation of past observations. How can we best work together across both sides of the Atlantic to increase our capacities for understanding and engineering biological systems at the genome scale?

The specific goals of the Workshop were to:

- Review and discuss the latest technical standards having to do with [a] vector architecture and physical assembly of genetic material, [b] *in vivo* measures of biological functions and parametrization methods/algorithms, [c] descriptive languages, graphical representations and formalisms for modelling genetic elements, and [d] data exchange supporting registries of standard biological parts.
- Present and discuss the emerging standardisation requirements needed to develop one or more *cellular operating systems* supporting genome-scale engineering.
- Identify and prioritise scientific research areas that would critically accelerate standards-driven biological engineering at the genome scale.
- Identify new research programs or resources needed to support the preceeding goals

Attendance to this Workshop was by-invitation-only and limited to ~24 internationally-renowned senior scientists. Yet, observers from the EU-US Task Force in Biotechnology Force were welcome and actively participated in the discussions.

As shown below, the Workshop included sessions devoted to the four goals mentioned above. Each session had a combination of invited brief presentations followed by a structured debate. Additional open discussion and breakout periods were also an important part of the whole endeavor. Participants who contributed to the discussion were expected and encouraged to serve later as local nodes back to their communities who might further the work of the relevant fields beyond the event itself.

The Workshop has produced three deliverables: **first,** a list of top standardisation *needs* not met or realised yet via current practice; **second,** a set of fresh scientific research areas that might be strategically coupled to support current engineering goals in Synthetic Biology; **third,** a working list of now unmet programmatic or facility needs necessary to see such work carried out in a timely fashion.

The Workshop was hosted by the Centro Nacional de Biotecnología, of the Consejo Superior de Investigaciones Científicas, and was held at the Parador de Segovia (Segovia

ES). All participants were accommodated at the same hotel so that interactions and scientific exchanges were ensured. The overall organisation of the Workshop was the responsibility of the EU-US Task Force on Biotechnology Research. The Directorate General for Research of the European Commission and the US National Science Foundation provided Administrative and financial support. Professor Drew Endy (Stanford University, USA) and Professor Victor de Lorenzo (Centro Nacional de Biotecnología, CSIC, Madrid, ES) were the Convenors of the Workshop. While a more detailed account of the various sessions is presented below, the basic conclusions of the encounter constituted a plea to foster a vibrant transatlantic action in the field.

ANNOTATED PROGRAM OF THE WOKSHOP

The event was structured around 5 sessions each devoted to one specific area. Each session included a combination of talks followed by a discussion that was recorded by a rapporteur in each case. The goal for each session was to identify tangible needs or next steps

Day 1. Fri June 4

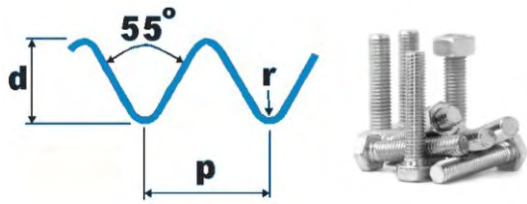
17.00h Collection of participants at the Meeting Point of the Terminal 1 of Barajas International Airport in Madrid

18.30-19.00h Arrival to the Parador in Segovia

19.00-20.30h Dinner

20.30-21.30h Welcome by the US-EC Task Force in Biotechnology, and briefing by the Convenors (D. Endy, V. de Lorenzo) on the Workshop, its objectives and its *modus operandi.*

Preliminary Discussion



The Whitworth thread. The ongoing discussion on biological standards get inspiration in similar ones that occurred along the pioneering times of modern engineering in the XIX century. The so-called Whitworth thread was world's first standard devised and specified in 1841. Until then, every industry had used their own screw threads. The new standard specified a 55° thread angle and a thread depth of 0.640327p and a radius of 0.137329p, where p is the pitch. The thread pitch increases with diameter in steps specified on a chart.

Following various seminal initiatives to have a comprehensive transatlantic discussion on the issue of standards in Biology, all attendants to this Workshop undertook the mission to address candidly all features around the issue. This involved not only identify bottlenecks and enumerate problems but, more important, to assess the state of affairs and propose joint avenues for action. Participants were aware of previous divergences on key issues between the SynBio communities at both sides of the Atlantic. But are such disparities necessarily undesirable? Quite on the contrary, having a global motion in Synthetic Biology, driven by at least two lively poles (US and EU) will make the field to move much more vigorously than having only one pole. At the end of the day, one is more certain on what time it is if one has (at least) two clocks instead of only one! Furthermore, the central question related to the development of *biological standards* can result only from the consensus between diverse stakeholders.

Day 2. Sat June 5

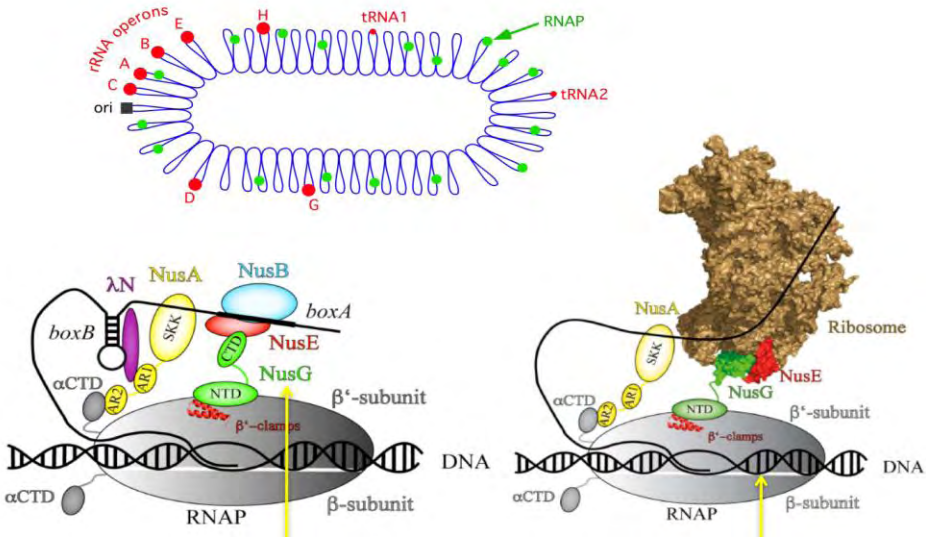
Session I **Functional composition of engineered biological parts and systems**
Chaired by: Víctor de Lorenzo
Rapporteur: Tim Ham

08.30-08.50 **Steve Busby**[1-3] (Univ Birmingham). Gene expression: from one promoter to one single cell to a whole population.
08.50-09.10 **Reshma Shetty**[4] (Gingko BioWorks, Boston). Promoters, RBS and coding sequences
09.10-09.30 **Christina Smolke**[5-8] (Stanford Univ). Riboswitches and other RNA elements: integration and insulation.
09.30-09.50 **Sven Panke**[9-12] (ETH, Basel). Metabolic blocks for engineering catalysts *á la carte*

09.50-10.30 DISCUSSION of Session I

Session I Summary and remarks

The key notion was how to bring in more engineering concepts and frames as to make functional composition easier. The challenge has to consider the many features of the prokaryotic RNA polymerase and the existence of transcription factories, which target the gene expression machineries into distinct compartments of the cells. A second aspect is RNA structure, the functionalities embodied in it and ways of entering signal-responding logic gates into the various isolated modules that can be built in RNA architecture.



Context-dependency of transcription. The cartoon sketches some of the cellular scenarios in which transcription occurs and which do influence the eventual outcome of promoter activity. First, genomic location (upper panel). It seems that the distribution of expression units is not random in the genome, but they obey to specific architectural constraints of the chromosome which direct given segments of the DNA towards transcription/translation factories. Second, the action of multiple termination /antitermination factors (lower left panel). Finally, the coupling of transcription with translation (lower right panel), which is mediated as well by a number of interactions between components of the corresponding machineries.

One challenging downstream problem is the engineering of orthogonal, context-independent translation signals upstream of output ORFs (GFP or other proteins) as well as the breakdown and subsequent reassembly of otherwise separated functional protein modules. Finally, engineering of central metabolic blocks may require an altogether different approach in which given enzymatic modules are separated from the rest of the network by means of proteomic switches. It is desirable that all these aspects and problems are tackled in a limited number of strains and model systems, which could crystallize in a Project that focus on development of key technologies. The main issue at stake is identification and eventual overcoming of the gaps that prevent integration of parts into a fully functional composition. Ultimately, it is believed that CAD (computer-assisted design) and DNA synthesis will overcome most of the current problems associated to these bottlenecks.

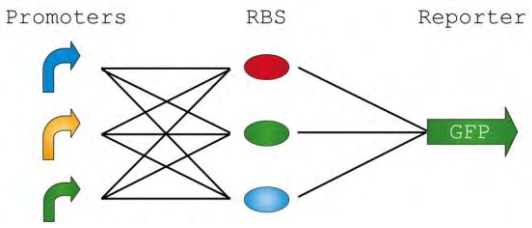
Session II **Quantification and measurement of biological functions**
Chaired by: Randy Rettberg
Rapporteur: Martin Fussenegger[1]

11.00-11.20 **Derek Wells** (Genencor, Palo Alto) Industry measurements: current practice and needs.
11.20-11.40 **Barry Canton**[13] (Gingko BioWorks, Boston). Measurements of engineered genetic devices.
11.40-12.00 **Richard Kitney**[14,15] (Imperial College, London). Large-scale characterization of biological parts and devices
12.00-12.20 **Drew Endy**[4,13,16,17] (Stanford, BioFab, Emeryville). Design and measurements towards a first expression operating system.

12.20-13.00 DISCUSSION of Session II

Session II Summary and remarks

Permanent requirements to tackling this subject include the need of agreed upon experimental models, ways to describe experimental data, and accessible databases. These should be more consistent, endowed with a more accessible format, richer in experimental details, and easier to search. To this end, the discussion identified an urgent need to shape a transatlantic working Group on standards, commissioned to implement various formats for increasing interoperability between models and model repositories, databases, and data recording/documentation. A side activity of such a commission would be to link to other interested communities e.g. Systems Biology. Note that different registries will serve different needs. The community needs to ensure free access to at least one registry which can be adopted as a community resource.



Contextualized measurements. One promising approach to develop compositional rules for expression devices is the generation of combinatorial collections of functional parts followed by the study of their recorded kinetic behavior as GFP output.

This resource needs well-curated parts and data. The proposed transatlantic Working Group would address and finalize the issues of parts processing standards, parts reporting standards, and criteria for *comprehensive* quality control. This is to be followed by a standard channel of short communications in scientific literature reporting the detailed characterization of the parts via distinct DOI (e.g. Symplectic Biology, http://www.symplecticbiology.org). Finally, the proposed Working Group would formulate two or more prototype cases that exemplify the IP problems specific to SynBio and present them to the competent bodies at the EU and US.

[1] Dr. Fussenegger could not attend the meeting due to disease. His slots were divided up between the other speakers of the session(s).

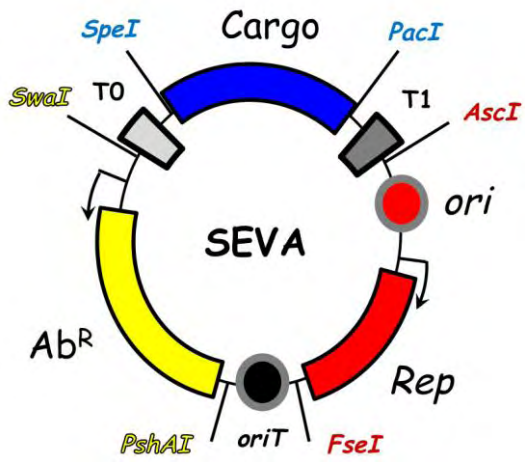
Session III **Physical composition of engineered biological systems**
Chaired by: Jörg Stelling
Rapporteur: Guillaume Cambray

15.00-15.20 **Ralf Wagner**[18-20] (GeneArt, Regensburg) State of affairs in *á la carte* automated gene synthesis: scientific and commercial angles.
15.20-15.40 **Víctor de Lorenzo**[21-24] (CNB, Madrid) The Standard European Vector Architecture (SEVA) initiative.
15.40-16.00 **Tom Knight**[4,25,26] (MIT, Boston). Current state of physical assembly standards.

16.00-16.30 DISCUSSION of Session III

Session III Summary and remarks

Physical composition is the basis for the definition of what a biological part is. Standards used for physical composition constrain downstream applications such as part characterization and measurement and devices behavior. Plasmids are a specific type of part/device used to propagate other parts: they should comply with the same standards. Physical composition is now a mature idea and several alternative techniques have been developed and experimented. We have good insights on the pros and cons for each of these techniques: [i] Gene synthesis: virtually no constraints; costly but dramatic improvement over the last decade and probably in the years to come; should be preferred if less expansive than conventional DNA manipulation techniques, [ii] Restriction enzyme based techniques: several standards are competing; leave scars in the assembled construct that can constrain function; cheap and efficient; automation-friendly, [iii] End joining: relies on PCR; leaves no scars and [iv] recombinering: mostly for chromosomal insertion; well defined techniques but standards are lacking. As of today, the number of entries in the MIT registry of standard biological



The Standard European Vector Architecture (SEVA). The essence of the SEVA platform includes **[i]** the anchoring of plasmid structure on 3 fixed insulator sequences that separate the three core functional modules, **[ii]** the formatting of the boundaries between such functional segments present in plasmids and transposon vectors, **[iii]** the editing/re-synthesis/minimization of each of these DNA parts for optimal performance in various prokaryotic hosts and **[iv]** the streamlining and simplification of their nomenclature.

parts and their level of characterization do not preempt a radical shift of physical composition standard. However, a number of initiatives aiming at massively characterizing parts are now emerging. As the type of physical composition technique used is likely to influence function of the resulting devices, it will be more difficult in the future to shift standards without wasting data. A working group could readily emerge to

define guidelines and good practices for physical composition. The goal would not be to define a common golden standard – as such a thing is unlikely to exist. The coexistence and ongoing invention of different standards is even needed and encouraged to address different context. Nevertheless, people working in the field should work together to ensure the compatibility (including backward compatibility, if applicable) between different standards. Such guidelines should clearly state which standard is preferable in which context (size of the part, potential interference of scars with biologically relevant processes…), and detail methods in such a way that the techniques would be accessible to inexperienced experimentalists. An online version of these guidelines would allow biological engineers to conform to good practices and facilitate the design of new standards that are compatible to existing one.

Session IV **Information exchange and language standards**
Chaired by: Christina Smolke
Rapporteur: Sven Panke

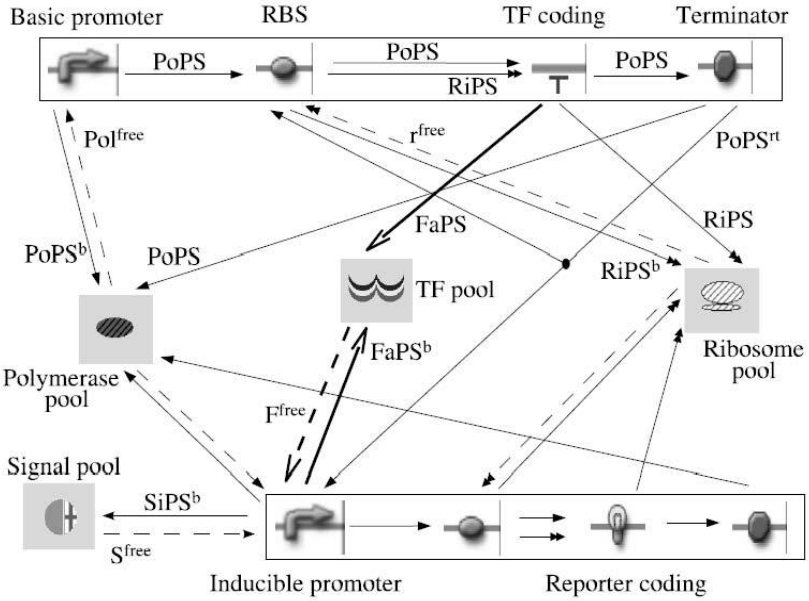
17.00-17.20 **Julie Dickerson**[27-29] (Ames, Iowa). Models and information management tools needed to support pathway engineering and cellular integration.
17.20-17.50 **Jörg Stelling**[30-33] (ETH, Basel) Composable mathematical models for synthetic circuit design
17.50-18.10 **Tim Ham**[34-37] (JBEI, Emeryville) Management and exchange of knowledge and information supporting synthetic biology research
18.10-18.30 **Randy Rettberg** (MIT, Boston). Electronic descriptions of standard biological parts.

18.30-19.00 DISCUSSION of Session IV

Session IV Summary and remarks

Computer-aided design, pervasive in other engineering disciplines, is currently developing in synthetic biology. Concepts for standardization and hierarchies of parts, devices and systems provide a basis for efficient bioengineering. Recently developed computational tools, for instance, enable rational (and graphical) composition of genetic circuits from standard parts, and subsequent simulation for testing the predicted functions in silico. The computational design of DNA and proteins with predetermined quantitative functions has made similar advances. The biggest challenge, however, is the integration of tools and methods into powerful and intuitively usable workflows-and the field is only starting to address it. Most existing platforms for circuit assembly are based on the *MIT Registry of*

Standard Biological Parts. Gene expression requires four kinds of signal carriers: RNA polymerases, ribosomes, transcription factors and environmental 'messages' (inducers or co-repressors). The flux of each of these types of molecules is a quantifiable biological signal exchanged between parts. These parts can be modeled independently by the ordinary differential equations (ODE) formalism and integrated into various softwares.



Computational tools for assembling gene circuits. Various platforms allow the possibility of easily visualizing and modifying DNA sequences of existing parts, and to analyze the behaviour of assembled circuits. Synthetic network simulators enables genome scale simulations considering RNA polymerases as signal carriers. These platforms must be improved to reflect translational and other post-transcriptional controls as well.

Drag and drop tools are most useful where genetic circuits are built just by placing biological parts on a canvas and by connecting them through 'wires' that enable flow of signal carriers, as it happens in electrical engineering. As SB circuits become more complex, other models and languages e.g. stochastic, will have to be considered.

19.00-20.00 Rapporteur & Session Chairs Working Session (leaders of each session to draft a one-page / one-slide summary of needs and opportunities)

Day 3. Sun June 6

Session V **Constraints and bottlenecks to standardization of biological engineering**
Chaired by: Richard Kitney
Rapporteur: Barry Canton

09.00-09.20 **Antoine Danchin**[38-40] (Ceprodi & AMAbiotics, Paris) Molecular traffic jams and emergence of metabolic and regulatory conflicts.

09.20-09.40 **Martin Fussenegger**[30,32] (ETH, Basel) Moving synthetic biocircuits from bacteria to animal cells.

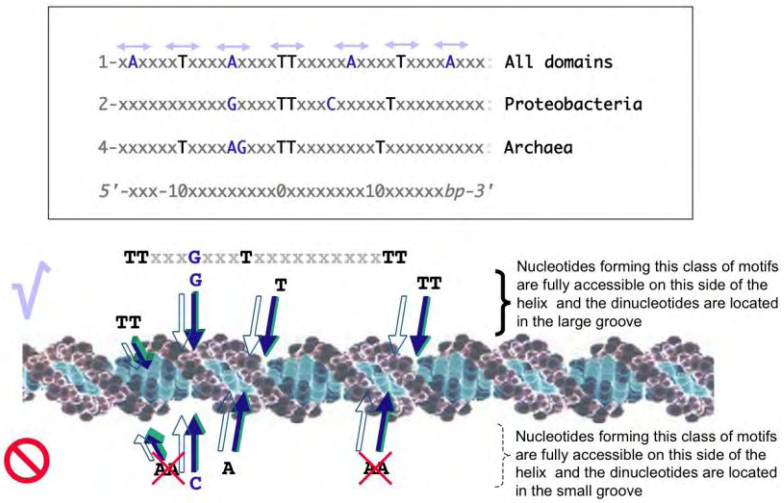
09.40-10.00 **Jim Haseloff**[41-44] (Univ Cambridge) Forward engineering of non-natural traits in plants.

10.00-10.20 **Francois Kepes**[45-48] (Genopole, Paris) Dealing with epigenetic phenomena in artificial genetic systems.

10.20-10.40 DISCUSSION of Session V

Session V Summary and remarks

All talks shared a common theme of addressing issues of engineering at levels beyond *simple* gene expression problems. These include metabolic jams, tri-dimensional genome organization, epigenetic effects, developmental signaling and morphogenesis. These aspects tend to be ignored but they frame and impose physicochemical constraints to any engineered system. One first case is that of the straight physico-chemical effects of given biological processes. For example, how do we address sudden changes in osmolarity due to changes in
inducer/substrate/product levels. The Lac operon illustrates a natural approach (LacA gene inefficiently converts and exports lactose, thereby preventing over-accumulation) with a strong takehome lesson re. the design of metabolic security valves to release over-accumulated metabolic intermediates.



Sequence periodicities found in DNA. Comparative analyses of the distribution of nucleotides along DNA sequences indicate that there is an inner logic in the location and frequency of various residues at different sides of the helix. Engineering complex systems might in fact be limited by
such non-trivial constraints which relate biological functionality with specific tridimensional architectures.

A second question is the role of the many highly-conserved proteins, some with known structure, but completely unknown function. But more bottlenecks do happen beyond metabolism. One of them is the control of the spatial distribution of the cell biopolymers (genome localization/compartmentalization, controlling the localization and conformation of mRNA), of which so little is known. In higher organisms, the problem extends into the signaling that controls cell geometry and organ demonstration, what leaves open various questions on the feasibility of morphogenetic engineering of the development and function of cells. Not to forget either that extant biological functionalities includes both genetic and epigenetic programs which are in some cases hidden in the available information (e.g. the localization of proteins is connected to codon usage). The main dilemma derived from these intricacies is whether SB can progress with a very imperfect knowledge of the systems which are to be engineered. Should we play with complexity or attempt to remove it to the best of our abilities?

11.00-13.30 **Session Reports and General Discussion of Outcomes** (chaired by D. Endy and V. de Lorenzo)

Workshop wrap-up

Although many activities that would now qualify as *Synthetic Biology* have been going on for some time in Europe and the USA (protein design, modelling, metabolic engineering, biological nanomachines), it is only now that the immense potential of the field is recognized as one of the most promising pillars of the sustainable and competitive economy of the future. All in all, SB is not about *understanding* Biological Systems but about *capitalizing* such systems as a source of components for creating new devices and properties to solve a variety of problems. In that respect, SB maps closer the realm of Technology, as long as it includes, but goes beyond basic Science –more interested to know and understand how existing Biological Systems work as they are.

Many European and US scientists still see it with scepticism that SB is a brand new field. In fact, there is a clear similarity between the discourse of Genetic Engineering of the late 70s and its many claims and the current assertions made by SB on its ability to solve problems in the future. But now, the international momentum provides a good opportunity for realizing the potential, finding a shared language and identifying synergies. This will require the development of much needed conceptual and material interfaces between the various subjects addressed in this workshop. Shared standards are a must in this context, as only they will articulate the synergies and joint efforts of the various SB communities across the ocean.

END OF THE MEETING

EXECUTIVE SUMMARY

STANDARDIZATION OPPORTUNITIES IN SYNTHETIC BIOLOGY

Standards can be used to coordinate work among people across time and place.

Standards can also be used to make regular materials and relationships among objects, physical or virtual, so as to enable reuse, composition into complex systems, and exchange.

Today, synthetic biology researchers have begun to explore and benefit from the use of standards in working together to make the process of engineering biological systems easier. These nascent standardizations efforts thus have the potential to greatly impact the underlying costs, times to completion, and chances of success for many existing applied biotechnology projects, from environmental remediation to drug and energy biosynthesis. Moreover, because advances in standardization can enable many people to work together on complicated large-scale projects, and can also support the integration of many components into complicated integrated systems, standardization via synthetic biology also has the potential to enable now impossible biotechnology projects.

Standardization via synthetic biology is often misrepresented as being only about the standardization of biology itself – that is, the physical *regularization* of the atoms and molecules that comprise life. While such an ambitious goal may ultimately be realized decades from now, the first *wave* of standardization in synthetic biology is focused on the coordination of activities among researchers, with immediate goals including to enable the sharing and reuse of genetic materials via **[i]** physical assembly and **[ii]** functional composition standards, **[iii]** measurements of cellular functions, and **[iv]** experimental data.

Modest but sustained public investments in both standardization research and research networks are essential to realize the potential of standardization in synthetic biology. However, care should be taken to ensure that such work does not become an end unto itself. Over and again, historical examples suggest that a multitude of standards can proliferate to no greater end without a driving large-scale project (e.g., the trans-Atlantic telegraph, or the human genome project), or without an internal community capacity sufficient to validate, promulgate, and support standards (e.g., the Seller's nuts and bolts machining factories). Moreover, nascent standardization efforts can also fall victim to preemptive institutionalization, bureaucracy, or politicization. Thus, complementary public investments in understanding the human practices of standardization would seem prudent, so as to help best guide early work and investments.

Opportunities via Physical Assembly Standards

Physical assembly standards in synthetic biology define how genetic materials, typically DNA, should be organized so as to enable more reliable assembly into ever-longer fragments. The purposes of such standards include [i] to reduce or eliminate planning costs and uncertainty in the DNA assembly process, [ii] to enable to organization, production, and redistribution of collections of readily reusable genetic materials, [iii] and to support automation.

The first widely adopted standard for DNA assembly (BBF RFC#10) was pioneered by Tom Knight of MIT and promulgated worldwide via the judging requirements of the International Genetically Engineering Machines (iGEM) competition. From a scientific perspective, BBF RFC#10 and other related standards *only* regularize the process of cutting and pasting DNA via a selected set of restriction endonucleases. Thus, a frequent criticism of such work is "there's nothing new here" given that basic genetic engineering methods have been in widespread use since the advent of genetic engineering a generation ago. However, such criticisms fail to recognize the purposes of physical assembly standards. For example, when a team of undergraduate researchers in Melbourne can make available genetic materials that can be instantly combined with the work of researchers in Barcelona, a significant technical advantage has been realized.

Technical shortcomings in the BBF RFC#10 standard have since been recognized supporting a modest flurry of additional proposals for physical assembly standards, most of which are publicly documented via the BioBricks Foundation (BBF) Request For Comment (RFC) process, which insures that emerging standards in synthetic biology are publicly disclosed and made available for widespread consideration, use, and comment.

Additional research and investments are needed to evaluate, validate, improve if needed, and promulgate a few best standards. Research should focus on standards that enable not just DNA assembly, but also RNA and protein composition. Further work should explore if physical assembly standards may also find practical use in PCR and recombination based approaches to the construction of genetic material.

A study should also be made of whether or not physical assembly standards will continue to flourish given ongoing advances in de novo DNA synthesis capacities. For example, one naive criticism of work on physical assembly standards is that, in the future, researchers will simply print all DNA they need from scratch, and thus there will be no use for methods the enable the combining of preexisting genetic material. A cursory response suggests such a future to be unlikely – for example, if DNA synthesis costs dropped by 100-fold over the next 5-10 years, re-synthesizing the top 1,000 standard biological parts would still cost US$10,000 (7,500 €) – but careful analysis seems warranted.

Opportunities via Functional Composition Standards

Functional composition standards in synthetic biology define how genetic materials should be designed so as to encode the expected behaviors when combined. By analogy and in building upon physical assembly standards, when a nut and bolt are physically assembled via regularized a screw thread, the fact that the nut does not then "fly" off the bolt when pulled upon represents a success of functional composition. That is, the composite object realized via physical assembly has the expected function.

Research on functional composition standards comprises and underlies the greatest challenges and promises of synthetic biology. Can we make the engineering of biology so reliable as to enable predictable behavior of many component genetic systems? Could we ever design and get to work an entire genome, and not just re-synthesize a preexisting natural sequence?

Practically, functional composition standards in synthetic biology are less well developed than physical assembly standards. One early example is to define the boundaries of "transcriptional "devices – composite genetic objects whose functioning is in part or whole realized via the process of transcription – so as to receive and send signals via a common signal carrier defined via the number of RNA polymerase molecules that pass a specific point on DNA per unit time (Polymerases Per Second, or PoPS). So defined, many genetic devices can be organized into a collection that supports functional interoperability via the PoPS standard. However, the PoPS standard itself remains incomplete, in that PoPS signal levels have yet to be regularized, PoPS levels cannot be measured directly, and reference standards supporting measurement and reuse (next section) remain under development.

Given the great potential and limited practice, it should not be surprising that there are more opportunities for research and investment in functional composition standards than can be listed here (or explored in a single meeting!). Very practically, broad areas of investment worth exploring and supporting now include **[i]** genetic and genomic layout architectures that increase the propensity for genetic elements encoding replication, transcription, RNA processing, translation, and protein processing functions to behave as expected when used in combination, **[ii]** genetic or genetically encoded linkers or insulating elements that can better combine or, instead, fully decouple biochemical functions encoded directly via DNA, RNA, or proteins, and **[iii]** basic research supporting regularization of relationships among intact cells, both natural and engineered, within the context of defined co-cultures or when known cells are combined with partially characterized natural ecosystems. Building upon such basic functional composition standards research, a next area of work focused on developing *higher-level* language and grammars for *programming* biology should be explored. Given that such follow-on work would be still less mature than basic functional composition standards research, it would be prudent to consider such research investments as being coupled to but not encumbered by work towards standardization.

Opportunities via Measurements of Cellular Functions Standards

Measurement standards define how objects and behaviors can be described and quantified by human beings or by human directed agents. Without measurement standards it is practically impossible to share results, coordinate work among parties, or to readily reuse materials over time. Thus, in synthetic biology, the development of measurement standards is absolutely essential to enable functional composition research, and must underlie any well defined and described collection of standard biological parts.

Drawing upon centuries of work from other fields of science and technology, measurement standards must at least include (i) a defined unit of measure, and (ii) a physical object that serves as a reference object encoding the so-defined unit of measure. Practically, it is equally important that surrogates of the reference object can be cheaply and accurately reproduced and promulgated widely and that, over time, any relationships between changes in the physical properties of reference objects and varying environmental contexts be established.

It is equally important to recognize that units of measure and reference objects are arbitrary in that they are human defined constructs. For example, the speed of light may be an unchanging physical constant defining something about the universe, but note that we define its magnitude via the units of "meters per second." Both meters and seconds are human defined constructs that are established, now with great sophistication, in relation to arbitrarily chosen physical reference objects. Thus, if the field of synthetic biology is to advance the standardization of measurements of cellular functions, it is important to recognize that we are likely embarking on a marathon (not a sprint) that will involve considerable bootstrapping.

Practically, via synthetic biology, one reference standard has now been developed and promulgated for assaying transcriptional activity inside living cells. This Relative Promoter Unit (RPU) standard relies on a synthetic transcriptional promoter against which the activity of other promoters can be compared. By making such comparisons researchers across disparate labs can reduce variation in reported measures of transcriptional activity from ~10-fold to ~2-fold, without having to standardize physical laboratory equipment, culture conditions, or other aspects of the measurement protocols. Such early work is notable for how it overcomes limitations found with earlier widely used methods for measuring gene expression, such as the Miller Unit, in which either reference objects or relative measurements frameworks were neither developed or promulgated.

Going forward, research investments should focus on the development of improved collections of reference objects supporting relative measurements across all major categories of molecular and cellular functions, including transcription, RNA processing, translation, protein procession, replication, and various aspects of metabolism. Additional research should focus on mapping the performance of reference objects across the range of contexts in which synthetic biological systems are likely to be used. As one simple

example, a reference promoter collection should be developed and modeled across the range of culture operating conditions used in industrial fermentation processes.

Opportunities via addressing knowledge gaps and overcoming biological bottlenecks

It would be naïve to consider that live systems can simply be broken down into a list of detached components and later rewired for a specific purpose -without surprises. While this would be ideal for engineering, the fact is that the functioning of virtually allexisting biological pieces seems to be context-dependent. Evolutionary pressure frequently results in a growing complexity of interaction networks and interdependence at all scales. Furthermore, proteins seem to possess an amazing ability to tinker with other proteins and to develop new interactions as soon as they are subject to selective pressure in a new host. A better conceptual frame is badly needed here to grasp what such minimal engineer-able biological building blocks are and how they can be defined or produced. While naturally evolved systems are still a phenomenal reservoir of biological activities that are in principle amenable to the type of forward-design that is at the core of Synthetic Biology, there are two standing issues that need to be tackled as a requisite for serious engineering of live systems/ objects. First, engineered biological modules, even whole organisms, should be made *orthogonal* (i.e., context-independent) in respect to existing counterparts. This would decrease the possibility of creating, unexpected emergent properties (the worst scenario for engineering) in the designed objects. Partial attempts in that direction involve the expansion of the genetic code based on reassignment of redundant and stop triplets or in the use of a 4 bp genetic code. One attractive possibility is to rely on xeno-nucleic acids (XNAs) as the information-bearing molecules, as they would be unable to talk to and exchange genetic material with its environment. There is still a long way to transform existing biological components, modules and systems into orthogonal equivalents, but the reward in terms of engineering will surely be enormous.

The second, related issue is the overcoming of the knowledge gaps -as well as cultural inertia that prevent standardization of biological parts and building blocks along the lines discussed above. Standardization of the elements and materials of globalized industrial engineering allows that the blueprint of a given object is conceived in Chicago, the parts produced in Mexico and the final assembly is made in Malaysia for the European market. The ones making the assembly do not need to know how the pieces were produced or designed. Is there anything similar that can be done with biological components? There are many ways of addressing this issue, all of them dependent on the final objective. As discussed above, standardizing cloning procedures is easy, as many restriction enzymes intrinsically produce cohesive ends that preset DNA segments for further ligations. Yet, even this simple opportunity of formatting is recurrently discarded. Every laboratory and every company produces vectors with completely arbitrary architectures and still more arbitrary names. There is an urgent necessity to have some sort of standards and fixed formats for vector organization and designation. This can result from adopting very simple agreements on the boundaries between functional elements and for vector nomenclature. Such an attempt was made at the beginning of the plasmid biology era, but it deafeningly

failed. Neither commercial interests nor the more or less anarchistic spirit of many molecular biologists (in contrast the more methodical engineering culture) seem to help much in this respect. The benefits of having just a few construction and assembly standards for DNA segments could be immense, but maybe the onset of cheap DNA synthesis (including complete genomes) will make the problem obsolete in the not so distant future. Still, for the next few years most biology laboratories will rely on manual procedures for cutting and pasting DNA.

A more difficult type of standards deals with basic biological functions, both in terms of measuring their intensity and predicting their performance. Transcription of one paramount case of this. Many synthetic gene networks involve transcriptional circuits) for which promoters with defined strengths and regulatable output are necessary. The issue of promoter power is not only inherently complicated but it is made still more thorny by the diversity of systems available to measure it. Reporter genes of all types (*lacZ*, GUS, *lux*, GFPs, *ina* etc) are used along with more direct procedures at the level of single cells. But the results are difficult to compare in absolute terms (let alone if one considers post-transcriptional effects). Would it be possible to have a conversion table by which the readout of each reporter system could be translated into conclusive counts of transcription initiation? For that matter, is transcription initiation a proxy robust enough for monitoring gene expression –and *vice versa*? The most recent work made on complete transcriptomes using deep sequencing of RNA reveals unsettling features on how simple bacteria manage *in vivo* their expression machinery. Abortive initiation, premature termination and stochastic binding of RNA polymerase to weak or cryptic promoters generates quite a diverse collection of transcripts from every gene, challenging the notion of the operon as a well-behaved polycistronic RNA-producing device. Is there then anything that can be done to produce transcription standards? There are at least two 2 challenges here: one is to use Systems Biology to understand the rules. The second is to exploit the rules for Synthetic Biology endeavours. But, in the meantime, the more one looks at actual data, the more we see failed attempts to be able to predict promoter strength and the degree of context-dependence of any given transcriptional module. Can we set reliable principles and actual activity units, given that we may still miss some fundamental facts on transcription control? A first attempt in the good direction could be the standardization of promoter strength measures that could at least give us an operative quantification of gene expression levels (see above). But what happens when one puts the same promoter in front of a different gene within a different genomic or physiological context? Many old-guard biologists would argue that things then become erratic. But should we then give up our attempts to have robust, formatted promoters with precise input/output specifications? This is an open question that lies at the core of Synthetic Biology. Perhaps we may once more return to Nature and find solutions there to this conundrum. For instance, viral (phage) RNA polymerases like the arch-famous one from T7 seem to have evolved precisely to work in a fashion considerably independent of the host's expression machinery. Given that the bulk of the genetic diversity of the Biosphere resides in the environmental virome, it may well happen that future synthetic constructs will rely on orthogonal building blocks (expression or otherwise) retrieved from bacteriophages rather than from bacteria or other fast-growing microbes. There is a considerable research agenda in sight before we reach a final conclusion to these matters.

Opportunities via addressing public perception

The ultimate agenda of Synthetic Biology is not just engineering for the sake biotechnology, but decisive understanding of Life as a distinct physico-chemical phenomenon. This obviously includes the question on the minimal genomes and the minimum components necessary for its emergence and its eventual recreation in the Laboratory. But, besides, the query embraces whether other forms of life and biological structures are possible, perhaps based on a different chemistry and principles of organization alien to what one could call *familiar Biology*. These questions have long left the realm of science-fiction and they are growingly becoming part of the public and private research agenda. Similarly to the onset of the genomic revolution of the last decade, these ongoing efforts re. *non-natural Biology* probably herald a change in our self-understanding and *Weltanschauung*, not devoid of ethical and societal angles. The *European Group on Ethics of Science and New Technologies* (EGE), which advices the EU President Manuel Barroso on this sort of issues adopted in Nov 18, 2009 the so-called Opinion 25 (http://ec.europa.eu/european_group_ethics/avis/index_en.htm) on the Ethics of Synthetic Biology. This advisory group goes beyond the usual precautions regarding safety and security of genetic engineering to state that *"… synthetic biology must respect the international framework on ethics and human rights and in particular the respect for human dignity…".* After 25 year of research and debate on genetically engineered microorganisms (GMOs, heavily sponsored by the EU: see http://ec.europa.eu/research/quality-of-life/gmo/). Many SB practitioners may wonder what is so offensive and morally alarming in the creation of artificial biological systems in the Laboratory. Paradoxically, the synthesis of equally artificial, xenobiotic pharmaceuticals which severely manipulate the natural course of biological processes (e.g. disease, aging) does not seem to raise comparable ethical concerns. One way or the other, the ultimate test of success of any new scientific ethos is whether it helps to move the knowledge frontier ahead and whether society benefits from it. Synthetic Biology is postulated to become a truly transformative discipline and the public perceptions of it at both sides of the Atlantic cannot be ignored.

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The four organizers of the Segovia Workshop. From left to right: Sohi Rastegar (NSF, US), Victor de Lorenzo (CNB-CSIC, Spain), Drew Endy (Stanford University, US) and Ioannis Economidis (EC, Brussels).

The Aqueduct of Segovia is one of the most significant and best-preserved products of Roman Civil Engineering. Researchers have placed its construction between the second half of the 1st Century CE the early years of the 2nd Century-during the reign of either Vespasian or Nerva. It has remained virtually intact for the last 2000 years.

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