

Series: iGEM Perspectives on Synthetic Biology

Scientific Life

Time to Get Serious about Measurement in Synthetic Biology

Jacob Beal,^{1,*}
Traci Haddock-Angeli,^{2,4}
Natalie Farny,³ and
Randy Rettberg^{2,4}

For synthetic biology to mature, composition of devices into functional systems must become routine. This requires widespread adoption of comparable and replicable units of measurement. Interlaboratory studies organized through the International Genetically Engineered Machine (iGEM) competition show that fluorescence can be calibrated with simple, low-cost protocols, so fluorescence should no longer be published without units.

Synthetic Biology's Evolving Measurement Needs

One of the less-often-appreciated differences between science and engineering is that engineering typically has much more stringent requirements for curation and replicability than science does. This is no criticism of scientific practice, simply a difference in goals and requirements. A scientific publication needs only to have data well enough curated to convince an expert human reader that observations and conclusions made with regard to a particular collection of experiments are likely to be valid. Effective engineering, however, has much stricter requirements for curation: typical engineering workflows require that many different sets of measurements taken by a variety of organizations be directly comparable, so that their informational and physical products can be reliably and predictably combined to create new artifacts.

Synthetic biology has been, in large part, a revolution driven by the application of the principles of engineering to the design of living organisms. Following the vision laid out early in its development [1], synthetic biology engineering can be thought of in terms of four layers of abstraction (Figure 1):

- production of DNA sequences with specified content;
- identification and design of DNA sequences to implement genetic parts such as promoters, ribosome entry sites, coding sequences, and terminators;
- composition of genetic parts into functional devices, such as sensors, logical operations, and actuators; and
- composition of devices to implement systems that achieve some engineering goal.

At this point, the ability to produce DNA sequences has become routine, supported by a competitive industrial market for DNA synthesis [2] and a sequence of improving methods for assembly (e.g., [3,4]), at least for sequences of moderate size and content. The identification and specification of parts is also well established: it is routine to identify and engineer promoters, coding sequences, recombination sites, terminators, etc. as well as to compose these parts into devices, as attested by the continued rapid growth of major public repositories such as Addgene and the iGEM registry of standard parts.

The synthetic biology community is now accumulating the preconditions for making the final step, to the routine and predictable engineering of systems: devices with strong signals, such as well-separated 'high' and 'low' outputs (e.g., [5–7]), methods to reduce the uncertainty in device composition (e.g., [8–10]), and models that can quantitatively predict certain compositions of devices (e.g., [6,11,12]). Such predictive models, however, remain quite limited and few if any systems have been

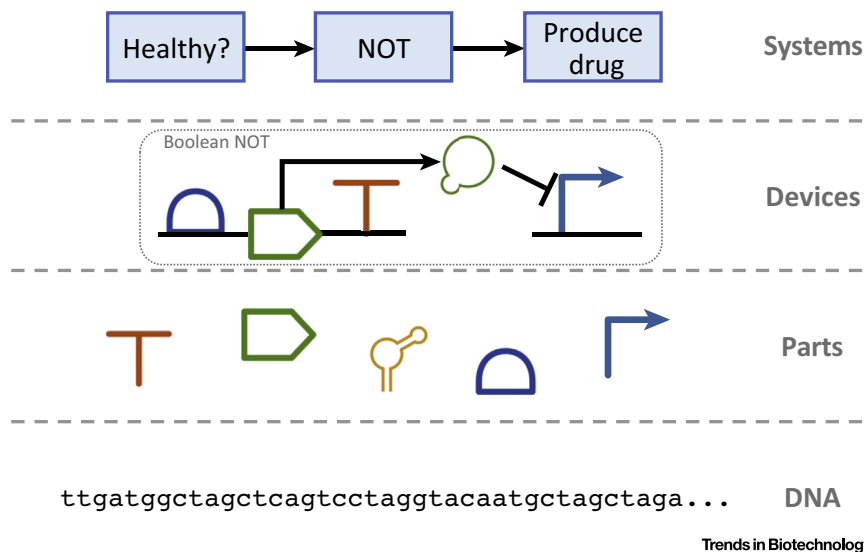
built that use them outside the laboratories that developed them.

The limited application of these models is diagnostic of a larger need for comparable and replicable measurements. Engineering of synthetic biology systems requires good quantitative models, and quantitative models cannot be effectively exchanged between organizations and applied unless those organizations use comparable and replicable units of measurement. Thus, the engineering of synthetic biology systems can never be routine until measurements are exchanged as freely and reliably between organizations as DNA, parts, and devices are now. In short, it is time to get serious about measurement in synthetic biology.

Comparable Measurements in the iGEM Community

Towards this end, the iGEM competition has been conducting interlaboratory studies on the measurement of fluorescence for the past 5 years. As an organization, iGEM is in a unique position to organize such studies: several thousand people, largely undergraduates and high-school students, participate in the competition each year and many have been eager to volunteer to help execute experiments in hundreds of institutions around the world. The iGEM interlaboratory studies also present a unique educational opportunity through which many of the graduate students, faculty, and industrial researchers of tomorrow can gain experience with the importance of comparable units and good measurement practices.

When we began the iGEM interlaboratory studies in 2014, we decided to focus on fluorescence as a critical problem area, since fluorescent reporters are widely used for quantification but are typically reported in noncomparable arbitrary or normalized units. To obtain a baseline for the degree of variation and replicability across laboratories, the 45 participating



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Figure 1. Synthetic Biology Engineering Can Be Viewed in Terms of Four Layers of Abstraction. As adapted from [1]: production of DNA, identification and design of genetic parts (e.g., promoters, coding sequences), composition of genetic parts into functional devices (e.g., a logical ‘NOT’ device where the production of a protein from one coding sequence represses the promoter that regulates another), and composition of devices to implement engineered systems (e.g., a genetic regulatory network comprising connected sensing, logic, and actuation genetic devices).

teams were instructed to measure the fluorescence of three test devices according to a supplied protocol and their laboratory’s best practices, in absolute units if possible. What came back was a remarkable *mélange* of different numbers and approaches to measurement, many not even qualitatively comparable due to the degree of difference between approaches and nearly all in arbitrary or relative units.

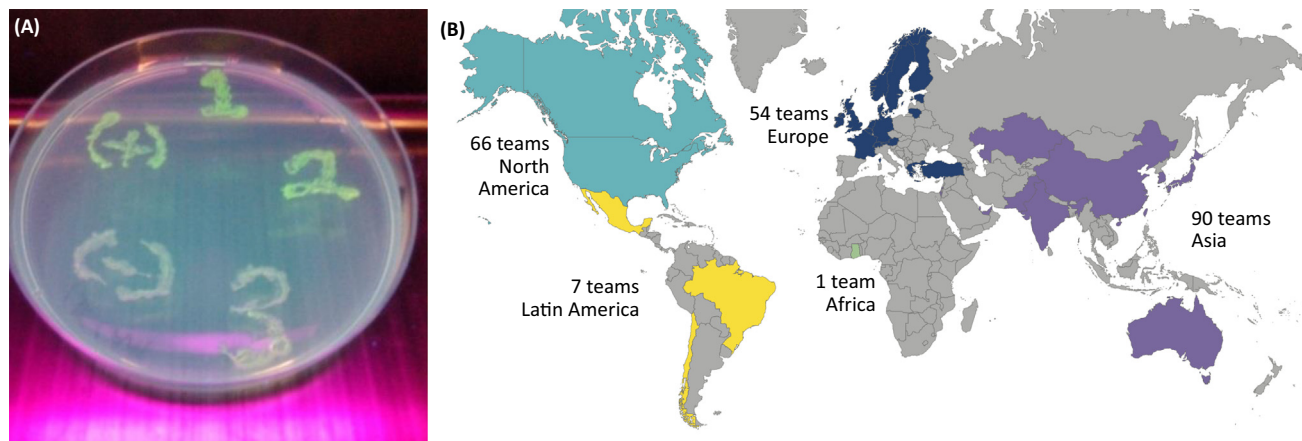
With this information, we designed the 2015 study, in which 85 teams participated, to focus on the most commonly available instruments (plate readers and flow cytometers) with positive and negative control devices and with a much more precisely defined protocol for measurement and reporting. By the end of this study, we still lacked comparable units, but we had discovered two significant and unexpected results [13]. First, ratios of fluorescence between highly expressed genetic constructs were quite precise (only approximately 1.5 times standard deviation in the ratios measured by different laboratories), while ratios of

high-expression to low-expression fluorescent constructs were highly imprecise (nearly sixfold standard deviation). Second, the dominant component of variation in measurement was not biological but tied to instruments: measurements of replicates were quite precise but instrument-to-instrument variation in ratios within a laboratory was much higher. In short, the challenges of reproducibility appeared to be rooted in problems with data acquisition and handling more than in difficulties with the cells themselves.

We viewed this result as good news, since problems in measurement should be easier to correct than some fundamental delicacy of biology. Thus, the 92 participating teams in the 2016 study were asked to calibrate measurements to comparable units against stable and affordable independent reference substances: fluorescein and LUDOX colloidal silica to calibrate fluorescence and absorbance in plate readers and SpheroTech rainbow calibration beads for flow cytometers (Figure 2). Given this calibration to comparable units, control

values could be used to systematically identify and exclude data sets suffering from protocol problems and the precision increased notably, even over ratiometric correction. The 2017 study replicated this success with more than 200 teams and the 2018 study focuses on refining the population estimates from absorbance using a bead calibrant to convert plate readers to the same MEFL units that flow cytometers are calibrated to with rainbow beads. Many challenges remain, but the interlaboratory study has moved the iGEM community from a condition of massive variation and incoherence in even the definition of fluorescence measurements to one in which hundreds of institutions can reliably obtain measurements with comparable units and less than twofold standard deviation.

The main goal of the iGEM measurement committee this year, however, is helping research groups at iGEM and elsewhere exploit these new measurement capabilities so they can more readily exchange data, debug protocols and systems, and advance the state of the art in synthetic biology engineering. Now that fluorescence measurements can be made reliably, one of the clear next questions is what measurements should be taken to enable routine composition of components. For devices in biological circuits, for example, at least the following need to be known: the maximum and minimum levels of steady-state gene expression, the input–output transfer curve (i.e., the expression level of the ‘output’ gene produced at varying levels of device ‘input’), and the signal-to-noise ratios (i.e., how large the difference between outputs expression levels is compared with the amount that expression levels vary between cells) [14]. Furthermore, most current devices do not have particularly good signal-to-noise characteristics, which means it is difficult to build circuits with well-distinguished output levels and that small changes in device behavior are likely to have large impacts on overall circuit



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Figure 2. iGEM Interlaboratory Studies Gather Data on Several Devices from Mean Teams Worldwide. (A) Fluorescence from *Escherichia coli* cultures transformed with positive control, negative control, and three test devices for the International Genetically Engineered Machine (iGEM) 2016 interlaboratory study (image supplied by iGEM 2016 team Macquarie Australia). (B) The iGEM 2017 study involved participants from 218 teams in 35 countries.

behavior [14]. As a consequence, much information is also likely to be needed on devices' interactions with their cellular context and one another. Many open questions remain, but they will be much easier to address with comparable and replicable measurements than without.

Building the Future of Biological Engineering

Given the state of the field and the results demonstrated in the iGEM interlaboratory studies, we conclude that measurement will become a primary frontier in synthetic biology development. Major advances in systems design are likely to emerge from interactions between organizations that produce and exchange data in replicable units, as more stringent curation and replication enable better debugging, better models, more effective devices, and engineering workflows joining the capabilities of disparate contributing organizations. The iGEM interlaboratory studies have shown that fluorescence can be cheaply and easily calibrated to comparable units and we plan to continue refining, applying, and disseminating that capability. If undergraduates and high-school students can make precise calibrated fluorescence measurements, there

is no reason that graduate students, post-docs, and professional technicians cannot do at least as well. Likewise, there is an important role for journals, editors, and peer reviewers to play, demanding fluorescence be reported in calibrated comparable units. Finally, fluorescence assays on plate readers and flow cytometers are only two of many measurement challenges facing synthetic biology, and as more are addressed the engineering capabilities of the field are likely to grow even faster.

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¹Raytheon BBN Technologies

²iGEM Foundation

³Worcester Polytechnic Institute, Worcester, MA, USA

⁴www.igem.org

*Correspondence: jakebeal@ieee.org (J. Beal).

URLs: <http://jakebeal.com>, <http://www.synbiotools.com>.

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