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Biological Control Variously Materialized:
Modeling, Experimentation and Exploration in Multiple Media

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This paper examines two parallel discussions of scientific modeling that have invoked experimentation in addressing the role of models in scientific inquiry. While the other discussion has considered the experimental character of models, the other one has focused on their exploratory uses. Although both discussions relate modeling to experimentation, they do so differently. The former discussion has considered the similarities and differences between models and experiments, addressing, in particular, the epistemic value of materiality. The focus on explorative modeling, in turn, has highlighted the various kinds of explorative functions of models in the early stages of inquiry. These two perspectives on modeling are discussed through a case study in the field of synthetic biology. The research practice in question explores biological control by making use of an ensemble of different epistemic means: mathematical models and simulations, synthetic genetic circuits and intracellular measuring devices, and finally electronic circuits. We argue that the study of explorative modeling should trace the ways different epistemic means, in different materialities, are being combined over time. Finally, the epistemic status of such novel investigative objects as synthetic genetic circuits is evaluated, with the conclusion that they can function as both experiments and models.

1. Introduction

The philosophical discussion of scientific models is undergoing a distinguishable turn towards practice and the epistemic aspects of the activity of modeling, and away from issues of representation. Two recent discussions, in particular, have attempted to flesh out the idea that models are objects with which we do something in our epistemic activities. While the one discussion has considered the experimental character of models (e.g. Cartwright 1999, Mäki 2005, Morgan 2003, 2005, Guala 2002), the other one has addressed their exploratory functions (e.g. Gelfert 2016, 2018, Massimi 2018, Fisher 2017). Both discussions relate modeling to experimentation, but they do so differently. The discussion of the experimental character of models likens them more directly to experimentation, highlighting those aspects of modeling and simulation that come close to experimentation. As a result, a new set of questions has emerged, concerning the ways modeling and experimentation resemble, or

differ from, each other, and whether those differences are due to the inferences they license, or the different role materiality plays in experimentation vis-à-vis modeling.

In contrast to viewing models as a kind of experiment, the interest in explorative modeling does not directly build on the analogy between modeling and experimentation. It rather takes the discussion of explorative experimentation as its starting point in focusing on the exploratory modes of reasoning characteristic of early stages of inquiry in which "an established prior body of knowledge" "cannot be assumed, or is itself at issue" (Gelfert 2018). Experimentation in such situations takes up the role of conceptual and theoretical exploration (Steinle 1997), as well that of stabilization of phenomena (Burian 1997). However, when applied to modeling, the idea of exploration gets new dimensions due to the fact that much of modeling is of a theoretical nature at the outset, and in many fields the theoretical activity consists predominantly of modeling.

Given these two parallel discussions of modeling, both inspired by the philosophical literature on experimentation, it seems interesting to discuss them side-by-side, in order to better understand the experimentable and explorative nature of modeling. The two discussions seem to place emphasis on different dimensions of modeling. The discussion of the experimental character of models concentrates on models themselves as workable, experimental objects, addressing in particular the epistemic value of materiality. The focus on explorative modeling, in turn, highlights the different uses of models in explorative activities. In order to better understand such explorative processes, we analyze (parts of) a research trajectory of one leading research group within the field of synthetic biology. Such a processual approach allows us to more fully understand explorative modeling. Another important aspect of explorative modeling that we wish to highlight is the use of various kinds of epistemic means – different kinds of models, experiments and measurements – that any genuinely explorative process typically involves. We submit that it is crucial not just to study single models, but also to focus on multiple models in multiple materialities.

The explorative process we will examine focuses on biological control, as well as on how scientists have combined different models of various materialities with others and with other epistemic means. The ensemble of different epistemic means used to explore the role of noise in biological organization consisted of mathematical models and simulations, synthetic genetic circuits and measuring devices, and finally electronic circuits. The status of synthetic and electronic circuits in this fabric is especially interesting. While they can be considered as experiments due to their material make-up, they can also be conceptualized as theoretical explorations. Especially synthetic genetic circuits lie squarely between modeling and

experimentation as they are constructed of the "same stuff" as the studied biological systems, yet are carefully designed making use of mathematical models as blueprints. Alternatively, the role of electronic circuits in this explorative process seems equally intriguing. Why did scientists, already armed with mathematical models, simulations, and synthetic genetic circuits built from genes and proteins, start to construct electronic circuits to study biological control?

Our case on biological control is based on a laboratory study of the Elowitz Lab at the California Institute of Technology, including its collaborations, especially with physicist Jordi García-Ojalvo at Universitat Pompeu Fabra and his co-workers. Michael Elowitz is the co-author of the Repressilator, which is one of the first synthetic genetic circuits to have been published, as well as probably the most famous one, due to its pioneering nature (Elowitz and Leibler 2000). There is already ample philosophical literature on the Repressilator (e.g. Knuuttila and Loettgers 2011, 2013), but the explorative process it gave impetus to, has neither been reported, nor studied. We will show how the process of exploratory modeling has established more conclusive evidence, now at the molecular level, for the role of stochastic fluctuations in biology. What is more, this evidence amounted to a redefinition of the target system: while human-made control systems are built to minimize noise, the scientists were able to make a convincing case that noise is an essential part of biological control.

We will begin with an overview on the philosophical discussion of the experimental character of models, turning then to explorative modeling. After that, we will consider the explorative research program on biological control and noise in which the Repressilator model has played a crucial role in its many incarnations.

2. Models as experiments?

Once models are couched as experimentable objects, it has proven difficult, at least on a general philosophical level, to clearly distinguish between modeling and experimentation. Although it seems intuitive to think that there are crucial differences between modeling and experimentation in terms of their respective targets, epistemic results or materiality, several philosophers have presented counter-arguments that largely bring modeling/simulation and experimentation together. The recent philosophical discussion has pointed out two ways in which they resemble each other. First, modeling and experimentation have been viewed as largely analogous operations aiming to isolate some core causal factors and their effects. The

argument is that both in modeling and in experimentation one aims to *seal off* the influence of other causal factors in order to study how a single causal factor operates on its own. Whereas in experimentation this sealing off happens through experimental controls, modelers use various techniques, such as abstraction, idealization, and omission as vehicles of isolation (e.g., Cartwright 1999; Mäki 2005).

One central problem of the isolationist view is due to the fact that idealizing and simplifying assumptions made in modeling are often driven by the requirements of tractability and mathematical convenience rather than those of isolation. (e.g., Cartwright 1999). This feature of mathematical models is further enhanced by their use of general, cross-disciplinary computational templates that are, in the modeling process, adjusted to fit the field of application (e.g., Humphreys 2004; Knuuttila and Loettgers 2012, 2016). Such templates are often transferred from other disciplines, as in the case of synthetic biology, where many models, formal methods, and related concepts originate from physics and engineering (e.g., the concepts of oscillator, feedback mechanism, and noise—see below).

Second, when it comes to simulation, numerous philosophers and scientists have pointed out their experimental nature as kinds of "numerical experiments" (Winsberg 2003)[1] Instead of isolation, the stress here is on *intervention*: both in modeling and experimentation one seeks to *intervene on* a system in the light of the results of this intervention. Consequently, simulations can be thought of as experiments performed on mathematical models. But the question is how deep does this resemblance cut. Two issues, in particular, have sparked discussion: the supposed target systems of simulations versus experiments, and the role of materiality they incorporate.

A common intuition seems to be that, whereas in experimentation one intervenes on the real target system of interest, in modeling one merely interacts with a model system (e.g., Barberousse et al. 2009). Yet, a closer examination has assured several philosophers that these intuitions may be deceptive. Winsberg (2009) argues that both "experiments and simulations have objects on the one hand and targets on the other, and that, in each case, one has to argue that the object is suitable for studying the target" (579; see also Guala 2002). Thus, both experimentation and modeling/simulation seem to display features of surrogate

[1] Another similarity between modeling and experimentation is related to data. Both activities produce data and deal with data analysis and error management (see Winsberg 2003, Barberousse et al.). We will not discuss this third commonality.

reasoning (Swoyer 1991), which is visible, for instance, in the experimentation on model organisms instead of the actual organisms of interest. Consequently, the closeness of a relationship of a model or experiment to its respective target need not distinguish the two activities from each other.

Even though modeling and experimentation appear to come close to each other, at the level of scientific practice we often do not have any difficulties in distinguishing model systems from experimental systems, although borderline cases do exist. Models and simulations are considered kinds of representations, and typically are expressed in other media than what their targets are made of, whereas experimental objects are supposed to share at least partly the same material make-up as the systems of interest. Indeed, the right kind of materiality has been claimed to be the distinguishing mark of experiments and even the reason for their epistemic superiority to simulations. Guala (2002) and Morgan (2003) have argued that the relationship between a simulation and its target is nevertheless abstract, while the relationship between an experimental system and its target is grounded in the same material being governed by the same kinds of causes. Consequently, while in simulation one experiments with a (formal) representation of the target system, in experimentation the experimental and target systems are made of the "same stuff." This difference also explains, according to Morgan and Guala, why experiments have more epistemic leverage than simulations. For example, anomalous experimental findings are more likely to incur change in our theoretical commitments than unexpected results from simulations (Morgan 2005).

Despite the intuitive appeal of the importance of the "same" materiality, it has been contested on different grounds. Morrison (2009) points out that even in experimental contexts, the causal connection with the physical systems of interest is often established via models. Consequently, materiality is not able to deliver an unequivocal epistemic standard that distinguishes simulation outputs from experimental results. Parker (2009) questions the alleged significance of the "same stuff." She interprets the "same stuff" to mean for instance the same fluid, and points out that in traditional laboratory experiments on fluid phenomena, many other things such as the depth of the fluid and the size, shape, roughness and the movement of any container holding it may matter. This leads her to suggest that it is the "relevant similarities" that matter for the justified inferences about the phenomena. Our case study on synthetic modeling shows that the "same stuff" was crucial in the study of genetic circuits. However, due to the complexity of intracellular mechanisms, and our scant knowledge of them, other kinds of models were needed that were triangulated with synthetic models in an explorative fashion.

3. Explorative modeling

The discussion of explorative modeling builds on the already established literature on experimental exploration (e.g. Steinle 1997, 1998; Burian 1997). Friedrich Steinle introduced the notion of explorative experimentation in his study of Ampère's experiments on Oerstedt's discovery concerning the influence of a nearby electric current on a compass needle. This discovery suggested that there is a connection between electricity and magnetism. Steinle's studies of Ampère's lab books lay out the methodological care with which Ampère proceeded. His endeavor was not based on trial and error, although no theoretical framework that would have explained the connection between electricity and magnetism was available. For example, Ampère realized right in the beginning that if he wanted to investigate whether there is a connection between electricity and magnetism, he had to isolate terrestrial magnetism from magnetism caused by electricity.[2] In order to separate the two effects, he developed a specific experimental setup. The case study shows that experiments do not have to be guided by an encompassing theory, since there are experimental guidelines such as systematic variation of parameters, formulation of stable empirical rules, and the exploration of which of the experimental conditions were necessary for the effect, and which were not. Moreover, such explorative experimentation can lead to conceptual developments especially in the early stages of inquiry.

Contemporaneously to Steinle, and in line with his findings, Burian (1997, 15) used the notion of explorative experimentation to characterize the "elaborate series of interconnected experiments" that were used to (re)identify and localize nucleic acids in the early phases of the study of these still ephemeral entities that are nowadays called mRNA and tRNA. The notion of explorative experimentation provided Burian an answer to the puzzle of how the work of the experimentalists in different groups and even in different disciplines could converge and stabilize around some shared 'theoretical' entities. The various procedures for experimental localization of entities provided explorative means for scientists that did not depend "wholly on the specific disciplinary or theoretical background of the experimenters, who initiated the work on those objects" (17).

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[2] Such separation between the two kinds of magnetism has a parallel in the case discussed in this article, where the scientists investigating noise proceeded to construct an experimental set-up that would allow them to distinguish between internal and external noise (see below).

Instead of trying to highlight the experimental features of modeling, the philosophers interested in explorative modeling have rather focused on some explorative features that are specific to modeling (although they may be found to apply to other epistemic practices as well). What the two discussions of exploration share, then, is the focus on the early stages of inquiry. However, Gelfert (2018) is careful to point out that the question is not just about the chronicle of events. Exploration seeks to get "a grasp of a phenomenon or scientific problem in the absence of a well-understood and workable theory of the domain in question" (4). He argues that this "early" character of exploration distinguishes it from heuristics or abduction. While heuristics is utilized in all stages of research, abduction, in Gelfert's construal, applies to later stages of inquiry, in which there already is a stable target phenomenon requiring explanation. But such a well-delineated target phenomenon cannot be taken for granted in the early stages of research. Indeed, one of the four exploratory uses of models that Gelfert puts forth is precisely that of "reassessing the suitability of the target".

The three other exploratory functions of models that Gelfert introduces are the uses of models as starting points of inquiry, as proofs of principle, and as sources of potential explanation. These uses are overlapping: often proofs of principle provide sources of potential explanation, and as such starting points of inquiry – the last one of these uses being so generally characterized that it does not carry too much analytical potential of its own. The functioning of models as proofs of principle and potential explanations has been commonly recognized in the literature of modeling – covering e.g. how-possibly, fictional and toy models – though the existing discussion has not addressed them collectively as various modes of exploration. Apart from providing the unifying notion of exploration for many so far separately examined activities of modeling, Gelfert's discussion of reassessing the suitability of a target system appears to us to break new ground. He studies such a process through Turing patterns, which present a case of a general mathematical model looking for suitable targets. Turing's model (1952) of the chemical basis of morphogenesis seeks to explain pattern formation in an otherwise homogeneous system through oscillations in concentrations resulting from the interaction between two chemical substances. The system consists of an "activator" that can make more of itself, and a quickly diffusing "inhibitor" that slows down the activator. This simple reaction-diffusion system is able to produce very complex patterns and might explain, among other things, spots and stripes in animals.

With respect to model-based exploration, especially three observations by Gelfert concerning the history of the Turing patterns seem particularly relevant. First, the biographies of scientific models are anything but linear, "models can go out of fashion and subsequently

rebound" (Gelfert 2018, 21). It was the better simulation techniques that sparked a new interest in Turing patterns, like in the case of many other simple mathematical models examining complex phenomena. Second, Turing patterns offered only a potential explanation and concerned, in Turing's own understanding, "imaginary biological systems". Such potential explanations are modal in nature; they target principles that might apply to a very wide range of different kinds of systems. Third, Gelfert mentions that apart from the availability of advanced simulation models, also their interplay with experimental manipulation was crucial for the revival of the Turing patterns.

We suggest that these two latter points are substantially intertwined. Namely, insofar as mathematical models study abstractly the dynamics of some general phenomena, and in doing so only provide potential explanations for particular real-world regularities or processes, the experimental grounding of these models in real phenomena becomes crucial. Such grounding does not amount to testing of the very general theory, as pointed out by the literature on exploratory experimentation. Rather, it situates the general model into the context of some real-world problem, and the existing knowledge concerning it, through an exploratory interplay of modeling and experimentation. In the following we will study exploratory modeling of genetic circuitry that made use of modeling and experimentation in various modes and materialities: mathematical models and their simulations, synthetic genetic circuits and measuring devices, and electronic circuits. Different models were constructed in different stages of the explorative process that led, much to the scientists' surprise, to the redefinition of the original target, biological control.

4. Exploration in biological control

Control in biological systems has been a central topic of biological sciences for a long time. Biological control is related to the high degree of organization in biological systems, from the molecular and cellular levels up to the organismic level, including properties that are considered to be biology specific such as their ability to maintain themselves, develop, and reproduce. Biological control became a subject of systematic study in the beginning of the 20th century. Françoise Jacob and Jacque Monod's (1961) discovery that gene regulation takes place via transcription factors was a milestone in experimental molecular biology. These findings were accompanied by theoretical studies making use of engineering principles, mathematical modeling, and general systems theory (Bertalanffy 1969). Early work in the 1960s on cybernetics (Wiener 1948) and information theory (Shannon 1948)

proceeded along the same lines. These different developments influenced the modeling of genetic and metabolic regulation in terms of feedback loops, where, for example genes are controlled at the level of transcription by the products of other genes. With the introduction of synthetic biology at the turn of the 21st century, it finally became possible to study such feedback systems within living cells. One of the pioneers of this approach is the Elowitz lab (Caltech), whose study of gene regulation extended into the examination of the role of noise in biological organization.

4.1 Mathematical and synthetic modeling in the exploration of noise

The research on noise in synthetic biology emerged from the question of how biological control in biological systems, such as in the circadian clock, could have been implemented (Loettgers 2009). Experiments on the circadian clock in molecular biology, as well as mathematical models by mathematical biologists like Brian Goodwin (Goodwin 1963) and Arthur Winfree (Winfree 1990) suggested that this kind of control is based on oscillations in protein levels. Michael Elowitz together with Stanislas Leibler constructed a synthetic genetic circuit, the Repressilator (Elowitz and Leibler 2000) that aimed to furnish a proof of principle that such oscillations could be produced by various kinds of molecular feedback systems.

The Repressilator consists of three genes that repress the protein production of each of its neighbor gene in the fashion of the rock-paper-scissors game. The mathematical model underlying the Repressilator is a system of non-linear coupled differential equations of the following form:

$$\frac{dm_i}{dt} = -m_i + \frac{a}{(1+p_j^{\,n})} + a_0$$

$$\frac{dp_i}{dt} = -b(p_i - m_i)$$

with: $i = lacI, tetR, cl$; $j = cl, lacI, tetR$

In this set of equations p_i stands for the concentrations of the proteins suppressing the function of the neighbor genes and m_i (where i stands for *lacI, tetR*, or *cl)* are the

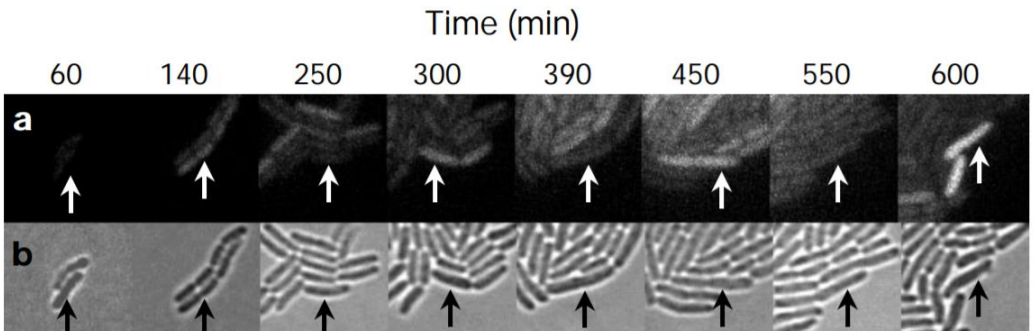
corresponding concentrations of mRNA. There are six molecule species (3 proteins functioning as repressors and 3 genes), each of them taking part in transcription, translation, and degradation reactions. In general, there are no analytical solutions for such non-linear coupled differential equations, and so Elowitz and Leibler performed computer simulations on the basis of this mathematical model. The main purpose of these simulations was the identification of relevant experimental parameters as well as the different possible states that could be exhibited by the system. There are two such states: a steady state, and a state in which the system performs limit-cycle-oscillations. Being interested in biological control, Elowitz and Leibler aimed for limit-cycle oscillations. For attaining these oscillations, the experimental parameters were of critical importance, and the simulations showed that such oscillations require, for example, strong promoters and tight transcriptional repression. This information was put to use in the construction of the actual genetic network, the Repressilator, for which the mathematical model functioned as a blueprint.

Although the computer simulations provided some important experimental parameters, most of the biochemical parameters remained unknown. The Repressilator did not seek to represent any naturally existing genetic circuit, it was a very simple construct made of well-characterized molecular components adopted from different contexts of research in view of obtaining robust oscillations. The same applies to the mathematical model that underlies it. Both models, the mathematical and the synthetic, can better be understood from the exploratory than the traditional representational perspective; they were purposefully constructed epistemic artifacts for probing *possible* architectures and dynamics of genetic circuits (see section 5 below for further discussion).

Another important exploratory dimension of the Repressilator was its implementation within a living bacterial cell that allowed the model to be explored in its natural biochemical environment. The actual genetic network was constructed by making use of a plasmid that was introduced into a cell. Plasmids are extra-chromosomal DNA molecules that are self-replicating. Figure 1 shows the architecture of the Repressilator and the interaction between the three genes.

Figure 1. The diagrams show sketches of the Repressilator and the reporter (Elowitz and Leibler 2000).

The genes of the Repressilator are connected via a negative feedback loop through which they repress each other's protein production. Green fluorescent protein (GFP) is used as a reporter and it is fused to the *tetR* gene. This construct makes the oscillations in the protein level of the gene visible through fluorescence microscopy. The entire construct, consisting of the Repressilator and the reporter, is integrated into *E. coli* bacteria. By its being constructed from biological components, and integrated into the bacteria, made the system more biology like. Although the biochemical interactions in the cell are largely unknown, this embedment, as Waters (2012) has lucidly spelled out, "avoids having to understand the details of the complexity, not by assuming that complexity is irrelevant but by incorporating the complexity in the models." The Repressilator was able to produce oscillations, but they turned out noisy (in contrast to what the underlying mathematical model predicted). Figure 2 shows the oscillations of the Repressilator, both in the growing bacteria colony, and in the diagrams depicting the measurements of oscillations in individual Repressilators.



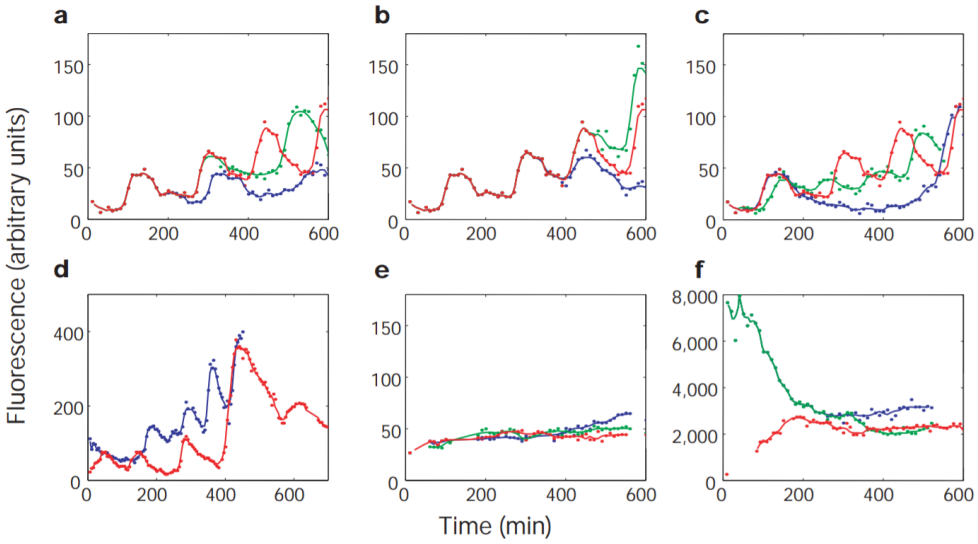


Figure 2. The upper picture shows the growing population of *E. coli* bacteria carrying the Repressilator. The lower picture shows the oscillations of single *E. coli* bacteria over time (Elowitz and Leibler 2000, 336).

The closer look at the pictures of the blinking bacteria colonies reveals that the blinking of the bacteria, i.e., the oscillations made visible by the reporter, are not synchronized. This non-synchronization is manifested even more obviously in the lower diagrams (a-c), showing the fluorescence of different sibling cells. Here the red line is a reference line representing the oscillations of the whole bacteria colony and the blue and green lines belong to oscillations of single sibling cells. The diagrams show that the amplitudes of the oscillations of the sibling cells change over time, meaning that there is a difference in the amount of proteins produced over time by the reporter gene. Secondly, the phase of the oscillations in the two bacteria shift over time. In other words, the sibling cells show some individual behavior (phase shift) but there is also some variability in this individual behavior (changes in amplitude). (The graph (d) presents oscillations obtained in different experiments, and (e-f) are the result of negative control experiments.)

The single cell measurements the Elowitz lab performed were laborious. One source of difficulties was due to choosing the 'right' parameter values. Elowitz explained this in the following way:

> [O]ne thing we've seen is that, the first generation of synthetic circuits were often overexpressing proteins a lot, and consumed a lot of resources and [that] often makes the cells grow a little bit slower than other cells that don't have the synthetic circuit […] it just means that maybe they're not optimized yet to be sufficiently independent […] the other side of it is that the environment inside the cell is not the environment of our model where there [are], you know, continuous variables and continuous trajectories.[3]

Another source has been the large apparatus consisting of microscopes, image processing utilities and computer programs that were brought into play, and adjusted for the analysis of the dynamics of the synthetic system. The complexity of this experimental set up contrasts with the apparent simplicity of the synthetic genetic circuit. Only when one focuses on the noisy character of the observed fluctuations, is one able to get a glimpse of the complexity of even such simple systems as the Repressilator.

The observed individual behavior of cells, shown in the phase shifts and fluctuations in Figure 1, provided a first clue that the fluctuations could be of a stochastic nature. Most probably, the researchers assumed, they were caused by the limited number of molecules in cells. In order to explore the noisy behavior exhibited by the Repressilator, the researchers performed computer simulations of a stochastic version of the initial mathematical model that seemed to confirm the stochastic nature of the observed fluctuations. Two related questions appeared: First, how are regular oscillations possible at all in the stochastic environment of a cell, and, second, how are stochastic fluctuations related to other sources of noise that occur independently from the observed stochastic fluctuations. Both questions were explored by further models and synthetic constructs. Of a particular interest is the synthetic intracellular measuring device that the Elowitz group developed to explore stochastic fluctuations by making them "visible" on a single cell level.

4.2 Measuring stochastic fluctuations in biological systems

A question, which was unable to be explored by the Repressilator, was the total amount of noise in a biological system. The total amount of noise is given by the sum of extrinsic and

[3] Elowitz in interview, conducted by Andrea Loettgers.

intrinsic noise. The Repressilator only provides insight into stochastic noise, which is part of intrinsic noise that has at least two sources:

- Individual messenger RNA does not get translated only one time during the transcription process, but can be translated many times, resulting in the production of many proteins instead of one.
- Promoters that regulate the transcription process by turning the genes 'off' and 'on,' can switch between long-lived 'off' and 'on' states.

As further experiments on stochastic fluctuations showed, both processes result in bursts of mRNA and, related to it, bursts of proteins reflected by the observed fluctuations in the protein level (Ingram et al. 2008, vol. 4). Intrinsic noise is closely linked to extrinsic noise. While intrinsic noise are fluctuations generating bursts of proteins, extrinsic noise are the *propagated bursts* of proteins affecting the expression and protein production of *other genes* in the system. There are various other extrinsic sources of noise that arise independently of the gene, yet act on it, i.e., the stage of the cell cycle, the mRNA degradation machinery and the cell environment fluctuations. Although extrinsic fluctuations are part of the dynamic and behavior of the Repressilator, they cannot be identified in its oscillations.

The basic motivation for constructing the synthetic intracellular measuring device was to distinguish between extrinsic and intrinsic fluctuations by separating them from each other (Elowitz et al. 2002; Swain et al. 2002). In constructing the measuring device Elowitz and colleagues made again use of *E. coli* bacteria. This time they integrated into the chromosome of the bacteria cyan *cfp* and yellow *yfp* alleles of green fluorescent proteins, which were then put under the control of identical promoters. In this set-up the intrinsic noise affects each of the promoters of the two reporter genes separately. The two genes are uncorrelated, meaning that the proteins produced by the genes fluctuate in an uncorrelated fashion (see the lower part B of Figure 3). This gives rise to a population of cells, in which some cells express more of one fluorescent protein than the others. As a consequence, the cells in the populations appear in different colors such as yellow, orange, red, and green.

In the absence of intrinsic noise at the two reporter genes, the two genes, which are located in the same cell, are only exposed to extrinsic noise that is the same for each of the genes. Consequently, the cells with the same amount of each protein appear yellow, as shown by the upper part A of Figure 3.

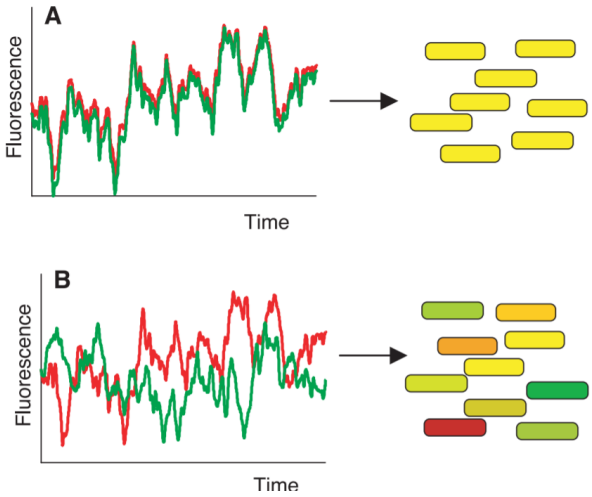


Figure 3. The fluctuations due to the extrinsic (A) and intrinsic (B) noise and the corresponding variations in fluorescence (Elowitz et al. 2002, 1185).

In an interview Michael Elowitz reflected on his expectations concerning the experiment:

> [W]hen I first was doing that experiment I really didn't know […]. There had been a lot of work theoretically, simulations, on how noise should be significant. But depending on the effective biochemical parameters inside the cell, which really were not known, and depending on how big the extrinsic noise is, it was hard to say whether the extrinsic or intrinsic effects would be stronger. I think what was interesting for me was, going into it, I really had no idea whether noise would just be a small perturbation on top of these big extrinsic fluctuations, or whether actually everything would be dominated by intrinsic noise. So, I think that was what was kind of fun about it […][4]

In exploring the relation of intrinsic and extrinsic noise under different conditions, experiments were performed by introducing the reporter genes into different strains of *E. coli*. The differences between these strains depended on how strongly the genes of the regulator sequences, to which the reporter genes were fused, were transcribed. By performing these experiments, Elowitz and co-workers were able to explore how the transcription of a gene is related to intrinsic and extrinsic noise. The result were differently colored colonies of cells

[4] The interview was conducted by Andrea Loettgers.

depending on the level of intrinsic and extrinsic noise (see Figure 4). Moreover, to study the interplay between the regulatory dynamics and noise, researchers introduced the Repressilator into one of the strains. From the results they concluded that changes in the regulatory dynamics may cause substantial changes in noise levels.

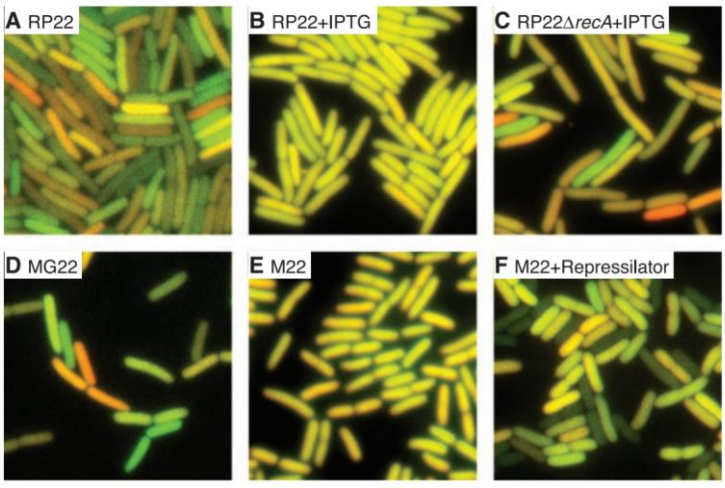


Figure 4. The pictures show the outcome of the experiments in which noise was explored under different experimental conditions by for example changing the reporter genes. (Elowitz et al. 2002, 1186). The last picture (F) shows the influence of the periodic dynamic of the Repressilator on the level of intrinsic and extrinsic noise in the cells (i.e. the contrast between pictures E and F).

These measurements were constrained by how much the parameters of the synthetic measuring device could be changed in a controlled fashion. Even this comparatively simple system turned out to be very complex and experimentally difficult to access. In addition to the Repressilator, and the measuring device for distinguishing between extrinsic and intrinsic noise, a third model was designed and used to explore noise in biological systems. The third system was not synthetic, instead it was an electronic version of the Repressilator. What could be learned from an electronic model about noise in biological systems?

4.3 The electronic Repressilator

Even though Elowitz and co-workers had been able to distinguish between extrinsic and intrinsic noise, and to explore them under different conditions, the question of how regular oscillations are possible at all under these circumstances still needed to be answered. To

answer this question an electronic Repressilator was constructed (Buldú et al. (2007). The electronic Repressilator was inspired partly by the work of Mason et al. (2004), who constructed an electronic circuit based on ordinary differential equations modeling a genetic network. Such an electronic circuit provides a good model for the study of robust oscillations, since, as the researchers put it "this system is subject to electronic noise and time delays associated with its operation, and since its parameters depend on the actual values of capacitances and resistors […]." (Mason et al. 2004, 709).

While Mason et al. (2004) constructed a hybrid digital-analog circuit, the electronic Repressilator of Buldú et al. (2007) is purely analog. Among the advantages of this approach is the experimental flexibility of electronic circuits as well as the possibility of constructing integrated circuits with a big number of elements. Figure 5 shows the layout of this electronic circuit. It is based on the same network design as the biological Repressilator consisting of "three dynamical elements coupled in chain with an inhibitory interaction" (Buldú et al. 2007, 3508).

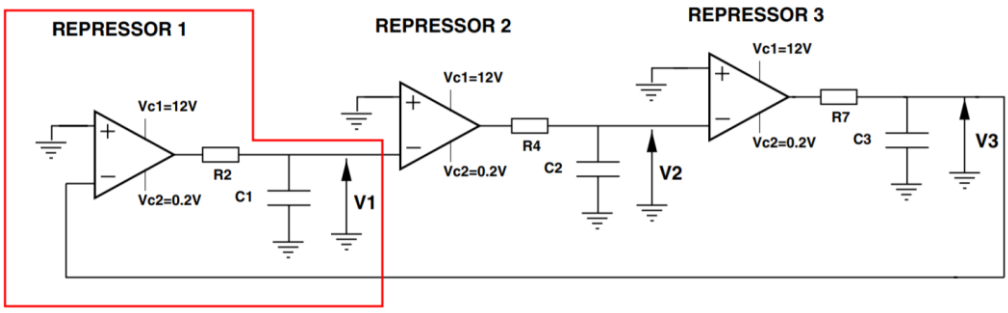


Figure 5. The diagram is a sketch of the electronic version of the Repressilator (Buldú et al. 2007, 3508).

The electronic version of the Repressilator is, as is the corresponding synthetic model, based on a mathematical model. It consists of three elements, each of them modeling a gene, which becomes repressed by the proteins produced by its neighbor gene. The three elements themselves consist of operational amplifiers and RC (R=Resistance C=Capacitor) circuits. The operational amplifier, designated as a triangle in the diagram, functions as a comparator and the RC circuit as filter. The voltages V_1,V_2 and V_3 are the analogues to the protein concentrations in the synthetic model. The dynamic of the electronic circuit can be described

in the following way: in case the voltage V_2 increases, it induces a reduction of the following output voltage V_3, which again lead to an increase of V_1. The model results in regular oscillations in the three output voltages of the electronic circuit, and is known in electrical engineering as a ring oscillator (displaying the engineering origin of the Repressilator) (Figure 6).

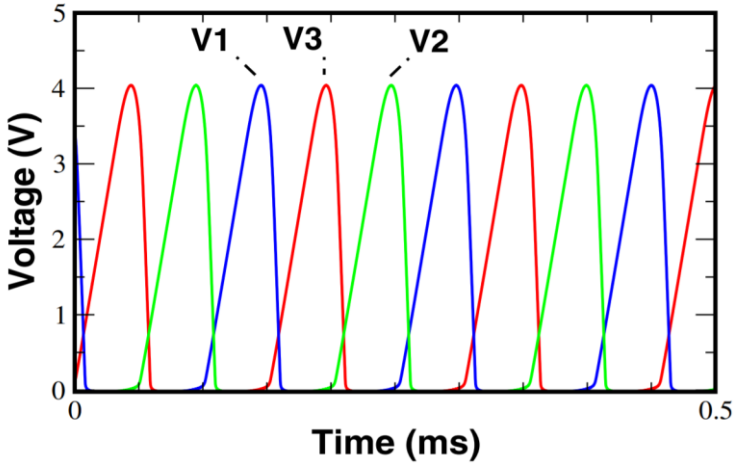


Figure 6. The diagram shows the oscillations in voltages in the electronic circuit (Buldú et al. 2007, 3508).

The electronic Repressilator shows that robust oscillations are possible despite of the presence of noise. But how could this result be transferred into the context of biology? The problem becomes one of relating the parameters of the electronic circuit to the genetic circuit, and it has so far remained unsolved. Accordingly, it seems that there are limitations to the widespread practice in synthetic biology of drawing analogies between electronic and genetic circuits. The electronic Repressilator turned out to be a valuable model in the exploration of possible network designs, however, due to its combination of experimental flexibility and implementation of noise in the electronic components. An example of this line of work is the interdisciplinary collaboration between Michael Elowitz, his long-time collaborator physicist Jordi García-Ojalvo and mathematician Steven Strogatz. They constructed a mathematical model of a population of Repressilators, coupled by quorum sensing (García-Ojalvo et al. 2004).

Quorum sensing is the common way of how bacteria communicate with each other by exchanging molecules. Following Strogatz's interest in synchronization of oscillatory systems such as the blinking of fireflies, García-Ojalvo et al. used the mathematical model

together with a computer simulation to explore conditions under which the coupled Repressilators would synchronize. Even though the model predicted that "a diverse and noisy community of such genetic oscillators interacting through quorum-sensing mechanism should self-synchronize in a robust way" (García-Ojalvo et al. 2004, 10955), the attempt of constructing a synthetic system of coupled Repressilators failed. Buldú et al. (2007) succeeded, however, in constructing an electronic version of the population of Repressilators that was able to exhibit synchronized oscillations. But given the problem of relating the electronic and biochemical parameters, they could not answer the question of why the synthetic system failed to synchronize.

To sum up, the Repressilator showed that it was possible to build, according to engineering and physical principles, a biological circuit able to oscillate. However, as these oscillations turned out noisy, the Elowitz lab went on to study the sources of noise, on the one hand, and the possibility of robust oscillations and the synchronization of oscillating bacteria, on the other. While the former studies were performed with synthetic systems, the latter were carried out with electronic circuits and mathematical modeling and simulation. Also elsewhere in synthetic biology a lot of effort was put into designing robust genetic circuits. Here especially the work of the Hasty lab at the University of California San Diego needs to be mentioned. Hasty and his co-workers were able to construct a robust oscillatory genetic circuit by paying close attention to the biochemical parameters, basically involving an unintended interaction between the synthetic genetic circuit and the host cell (Cookson et a. 2009, Stricker et al. 2008).

Finally, Elowitz and his co-workers also addressed the role of noise in biological organization. In several studies, the members of the lab showed that noise is not a nuisance in biology but has a functional meaning (Süel et al. 2007; Long et al. 2008; Eldar and Elowitz 2010). Noise in the form of stochastic fluctuations triggers as well as controls processes in cells. In addressing the functional meaning of noise, the Elowitz lab drew inspiration from the study of neurodynamics and lasers, mentioning also the classic experiments by Spudich and Koshland (1976) on nongenetic variability of the bacterial chemotaxis. Spudich and Koshland had concluded that "nongenetic variability would be a preferred mechanism for accommodation to random fluctuations in the environment and genetic variability the preferred mechanism for accommodation to long lasting environmental changes" (1976, 470). With the benefit of new technologies, the Elowitz lab was able to study such nongenetic variability in the form of stochastic fluctuations within living cells. Importantly, their

exploration on noise led to a development of an experimental-cum-conceptual framework in which noise and control became intertwined and redefined.

5. Discussion and conclusions

The explorative research program on biological control and noise by the Elowitz lab and its collaborators portrays an intricate fabric of mathematical modeling and simulation, intracellular measurement, and the construction of synthetic genetic circuits and electronic circuits. We deem multiple modeling and the combination of different epistemic means a central scientific practice that merits more research from the exploratory perspective. That contemporary modeling practices typically employ multiple models, and triangulate them with other epistemic activities is something that has been recognized and analyzed by the philosophical discussion surrounding robustness. However, robustness analysis has been focused on convergence; on either the use of multiple related models to causally isolate a core mechanism producing certain phenomena (Knuuttila and Loettgers 2011), or on the use of independent epistemic means (models, experiments and observations) to "triangulate the existence and character of a common phenomenon, object or result" (Wimsatt 2007, 43). Although both of these aspects of robustness analysis are present in our case study, the notion of explorative modeling better captures how the Elowitz lab and its collaborators studied biological control, and noise. In line with robustness analysis, the scientists were using multiple, materially distinct and thus partially independent epistemic means, in order to study whether the simple network design could create robust oscillations at the molecular level. However, this triangulation process is better conceived of as providing an initial proof of principle, in the form of the Repressilator model that then led to further exploration into the sources and role of noise in biological control. In this explorative process, materially different models were used rather in a dialogue with each other than only as an attempt to isolate a core causal mechanism common to all of them. In contrast, the models studied were different material realizations (digital, synthetic and electronic) of a particular circuit design.[5]

Massimi (2018) discusses multiple models as means of exploration, and casts, moreover, explorative modeling in a modal idiom. She presents a case in Beyond Standard Model (BMS) physics, where different models perform the exploratory function in charting and carving out the space of possibilities (349). The Elowitz lab's research on biological

[5] The epistemic importance of the different materialities and formats of models has also been studied by e.g., Chandrasekharan and Nersessian (2011), and Vorms (2008).

control was also importantly modal in that it was motivated by the question of whether the kind of feedback systems familiar from physics and engineering already theorized since the early 1960s (e.g. Jacob and Monod 1961*)* could be realizable in biological organisms. Synthetic biology finally gave means to scientists to study such *possible general design principles of biological organization* within living cells. The underlying additive character of Massimi's "perspectival modeling" does not easily accommodate our example, however. In her construal, perspectival models provide each a partial account of the phenomenon of interest, where the "exploratory function is performed jointly" (350). The multiple modeling process of the Elowitz lab had more of an unfolding character. Their models and measurements functioned as kinds of target systems/ research objects for each other in the investigation of the entangled, and largely unknown phenomena of noise and control. In this process the phenomenon of interest, biological control, became reassessed, as researchers realized that noise might play a crucial role in it.

Finally, the synthetic biology program of the Elowitz lab provides insight into the discussion of models as experiments. The construction of synthetic constructs – synthetic genetic circuits and synthetic intracellular measuring devices – each functioning within individual, and multiplying, bacterial cells, provides the particular novelty of the research practice of the Elowitz lab. For considering the experimental character of models, synthetic genetic circuits seem especially exciting, as they seem difficult to place either in the category of modeling or that of experimentation. The comparison of synthetic genetic circuits to the electronic circuits on the one hand, and to the synthetic intracellular measuring devices on the other, helps to identify the model-like and experimental features of synthetic genetic circuits.

First, while both electronic and synthetic genetic circuits are material model systems used experimentally to study biological control, we seem more inclined to treat such electronic circuits as models than synthetic circuits. Why? The answer appears to be that because electronic circuits are implemented in another medium than the biological systems of interest, they are more readily called models. For example, Rheinberger (2015) distinguishes what he calls 'preparations' from models on this basis: while preparations "participate in the materiality of the object of knowledge in question", models do not (325). We do not consider this a very substantial point. Both electronic circuits and synthetic circuits can be considered models in that they are tightly constrained and (to a degree) self-contained constructions designed to explore certain pertinent theoretical and empirical questions (Knuuttila 2011, 2017). Neither of them aims to *re*present some *particular* naturally occurring target systems, they rather present and study very rudimentary and highly hypothetical design principles,

tentatively applying to actual and possible non-actual biological systems alike. Such program is in line with the grander vision of synthetic biology: "the expansion of biology from a discipline that focuses on natural organisms to one that includes potential organisms" (Elowitz and Lim 2010, 889). This modal character of synthetic biology is precisely one of the most important reasons for approaching it from the explorative perspective (Knuuttila forthcoming, Knuuttila and Koskinen Gelfert 2016, 2018).

The comparison of the Repressilator to the synthetic measuring device, the noise sensor, further underlines its model-like character. In contrast to the Repressilator, the noise sensor was not constructed to fulfill some specific biological function and, consequently, to exhibit at least partially independent behavior from the rest of the cell. In designing the Repressilator, in contrast, the scientists aimed for a synthetic module that would, by being able to create new behavior, provide a tool for the exploration of possible design principles of biological organisms (see above). The noise sensor as opposed to the Repressilator did not have a dynamic of its own. The independent functioning of the Repressilator was crucial for its role as an exploratory tool, whereas the noise sensor was implemented as an integral part of the cell, and was supposed to be responsive to various conditions taking place in it. Thus, although the Repressilator and the noise sensor were both parts of the cell, they were considered different kinds of things with only the former having a proper model-like character.

Yet, at the same time, the material make-up of the Repressilator, its being of the same stuff as the naturally occurring genetic circuits, and furthermore implemented in the natural cell environment, gave it a highly experimental character. As such it was exposed to the (largely unknown) constraints of naturally evolved biological systems that explains how the researchers reacted to the unexpected results. The irregular oscillations were taken more seriously than what any particular results derived from mathematical models would have been, spawning instead a new research program on noise. The fusion of the model-like and experimental features of the Repressilator speaks in favor of considering it from a broad explorative perspective. There is no need to dress such human-made epistemic artifacts as synthetic genetic circuits necessarily in either the experimental or modeling guise, as they can function in both modes, even simultaneously.

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