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Exploration, iterativity and kludging in synthetic biology

Maureen A. O'Malley

Egenis, Byrne House, University of Exeter, St Germans Road, Exeter EX4 4PJ, United Kingdom

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ABSTRACT

Synthetic biology is the latest manifestation of post-genomic practice in molecular biology. It involves the engineering of parts and systems, and is often declared to be a practical 'proof' of mathematical models and design strategies. By examining the range of practices that constitute synthetic biology, a broader philosophical understanding of the molecular life sciences can be developed. Rather than focusing on hypotheses, testing and inference, synthetic biology invites attention to the practices of exploration, iterativity and kludging. Examining such strategies in relation to synthetic biology offers new avenues of insight for philosophy of science and biology.

1. Introduction

Philosophers of science and biology are these days as least as interested in biological practices as they are in theory and methodological principles. This is in part because recent trends in molecular life sciences do not seem to be captured easily by formulations about hypothesis testing and strict experimental design. Close attention to the activities of contemporary molecular biologists and other scientists is thought of as an effective way of gaining philosophical understandings of their scientific achievements [1–5]. My suggestion in this article is that a number of general modes of practice provide alternative philosophical spheres of investigation to hypotheses, testing and inference making. Broader activities within which many aspects of molecular life science can be understood include the practices of exploration, iterativity and kludging. I will sketch out each of these dimensions in relation to an emerging form of molecular biology, synthetic biology.

1.1. Synthetic biology

Throughout much of this article I will talk about synthetic biology as if it were a distinctive and coherent set of practices. In practice, however, there are a number of different streams in synthetic biology, and each stream produces knowledge somewhat differently according to a variety of philosophical orientations (see [6] for details). The first stream is one that I and my colleagues have described as DNA-based device construction. It begins with DNA synthesis and works upwards to the construction of parts, devices and, ultimately, systems. Standardization, decoupling, and abstraction are invoked as crucial aspects of biological construction [7–9]. These activities, aimed at achieving more complete control of biological processes, are seen as part of the 'decomplexification' of biology [10–13].

The second stream of synthetic biology can be characterized as genome-driven cell engineering. These synthetic biologists streamline and modularize genomes through techniques of minimal genome analysis, whole-genome synthesis and genome transplantation [14–21]. The genome is treated as a plug-in programme module that runs cellular processes. The creation of a simplified cell 'chassis' is seen as a desirable product and tool by this and other streams of synthetic biology [22,23].

The third stream of synthetic biology aspires to create protocells, using existing and modified biological parts such as micelles, self-assembling lipids, vesicles and ribosomes [24–29]. These practitioners engage in top-down, bottom-up and in-between approaches. Their primary aim is the

E-mail address: M.A.O'Malley@exeter.ac.uk.

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construction of minimal or minimized cells that can function as basic approximations of living cells [30]. Protocell synthesizers acknowledge the evolutionary complexities of life, but they too wish to diminish such complications in order to reconstruct life more effectively.

Bringing all three categories together again means we can view synthetic biology over time and see how a 'first wave' of construction of very simple parts and modules is leading to a newer second wave of whole system construction [31]. This ambition (and potential achievement) is shared by all streams of synthetic biology. Another characteristic that unifies the field at least at an abstract level is the drive to replace or displace complexity with rationally determined, highly predictable systems. This does not mean that there is no recognition of biological complexity by synthetic biologists — quite the opposite, in fact. But, in order to achieve the goal of constructing designed and decomplexified systems, synthetic biologists believe that engineering of some sort is inevitable [32,33]. They argue that engineering approaches will work only if the excess or irrational complexity of living systems is minimized or, better, avoided altogether.

> "The overwhelming physical details of natural biology... must be organized and recast via a set of design rules that hide information and manage complexity" [34].

Through cycles of design, construction, trial-and-error tinkering, redesign and reconstruction, synthetic biologists have produced some remarkable biological devices. These are frequently built on the basis of transcriptional regulation [35–37] but also use other biological processes [31,33,38,39]. Just as interesting as these constructions are the epistemic routes synthetic biologists have to take to achieve such goals. Rather than hypothesis testing in a formal and narrow sense, synthetic biology invokes a range of philosophically neglected strategies for gaining scientific knowledge. I will characterize these as exploration, iterativity and kludging. They are not by any means sequential, but I will have to describe them serially to make that obvious.

2. Exploration

Many scientists and some philosophers believe that true science is hypothesis-driven (HD) and that scientific claims to truth must be based on tests of hypotheses [40,41]. Descriptive, inductive or generally exploratory methodologies are conceived of as fundamentally preparatory exercises that pave the way towards 'genuine' HD science. Despite the commonness of this view, a great deal of evidence from earlier and recent science indicates that it is a misleading one [42]. The histories of diverse achievements in physics, chemistry and biology suggest that accounts of scientific practice should involve not only the formulation and testing of hypotheses but also the exploratory investigation of phenomena, in which investigation may be driven by a range of factors such as data gathering, technology development, or general questions directed towards an understanding of the scope or nature of the research domain [43–48].

An examination of the history of microscopy, for example, demonstrates how the development of technology can lead to a major reorientation of background assumptions and a plethora of ongoing research questions [49,50]. The exploratory investigation of regularities amongst microscopic phenomena led both to application-oriented medical research and to fundamental questions about the nature of life and its generation [51]. The history of chemistry provides a similar picture, in which general questions and the exploration of new technologies were major forces in the development of the discipline and its scientific achievements [46]. Biology also offers a plenitude of such examples, including the major milestone of Charles Darwin's theory of natural selection. Although some commentators have enthusiastically reconstructed *The Origin of Species* as a product of hypothesis testing [52–54], for many other observers it is clear that Darwin spent over two decades in a highly exploratory mode of identifying regularities in several biological domains, and classifying the phenomena to be explained [55,56]. More recent examples can be found in contemporary molecular biology, where high-throughput data acquisition and technology-driven developments all indicate the importance of a diversity of non-HD practices [48,57–60].

Synthetic biology, which has arisen out of genomics, is also a field that is not easily captured by a linear model of hypothesis testing. Instead, it exemplifies a range of exploratory modes of investigation, in which design space is explored and reconfigured, questions about phenomena are proposed and probed, and technology is developed to engage with novel or recalcitrant phenomena [61]. A very illustrative example of exploratory strategies comes from the study of biological noise, which refers to the random fluctuations in cellular processes produced by inherent molecular stochasticity. Noise is generally conceived of as the opposite of signal, and it is something usually minimized or ignored through various accommodations of the models used to understand the 'real' phenomena. But in synthetic biology noise is being examined as a phenomenon and used as a tool in its own right. Exploratory studies have probed noisy phenomena and categorized types of noise according to their sources and effects [62–65]. Further investigation has focused on tentative descriptive models of noisy processes, with the aim of being able to predict and harness their outcomes [12,66–68]. Engineered devices, such are genetic switches and oscillators, have been built on the basis of noise and stochastic processes in general, and these devices further explore the phenomenal space of cellular interactions [35,69]. Totally new combinations of technologies are being developed to study noise effectively [70]. These exploratory studies are now fundamentally changing the conceptual framework of what noise and information are in biology, with emerging understanding of how noise affects or even effects developmental mechanisms and robustness to environmental perturbation [71–76].

The noise biology example and the briefer ones above it provide a basis for a sketch of what exploration entails in scientific practice. One obvious characteristic is that inquiries tend to anastomose as successive investigations produce partial answers that modify the original aim of

inquiry, shift or tighten its focus, and generate additional interconnecting lines of research. Although such practices can be described as exploratory, this does not mean they are preliminary or undirected. The term simply indicates that such investigations do not have pre-ordained endpoints. Nor is exploration loose or vague. Exploratory work quite commonly involves the systematic variation of precisely defined parameters. It searches for regularities, and seeks to individuate, characterize and quantify previously unknown or neglected phenomena [59,77,78,79,80]. Noise is just one example. This sort of exploration occurs in conjunction with question-driven inquiry, which may expand the original domain of inquiry, or collapse it into a focal point that is then amenable to narrower hypothesis testing [48,81]. Another aspect of exploratory investigation is its orientation to technology, which may involve the development of entirely new instruments and techniques, or the modification of existing tools for novel contexts [43,79,82]. Various combinations of exploratory modes of scientific practice may enable the reconfiguration or replacement of existing conceptual frameworks, as the noise example showed.

Unlike HD science, broader exploratory practices are often not contained within disciplinary boundaries, due to ways in which they reconfigure technologies, theories and disciplines. Systems and synthetic biology are explicit about their intent to cross and transform such boundaries. But despite the major differences between HD and exploratory modes of investigation, they should not be understood as dichotomous or mutually exclusive categories of practice. Hypothesis testing functions as a highly specialized interrogative practice that works only when the context of inquiry has been sufficiently delimited. The standard representation of science as HD is typically based on situations in which very specific questions can be addressed within bounded spheres of inquiry and disciplinary contexts [43,83,84]. Generally, exploratory work is required to generate the detailed understanding and background knowledge associated with such contexts, and then to take narrow hypotheses meaningfully forward again. Most scientific inquiry will, therefore, consist of a range of investigative modes — a 'methodological toolkit' [85]. This toolkit may include hypothesis testing at some points, but it occurs within the context of broader modes of investigation that are in ongoing interplay with one another. This iterative interaction is crucial to the scientific process.

3. Iterativity

Iterativity is frequently mentioned as an inevitable element of practice in synthetic biology [31,86,87]. It is not, however, much discussed in the history and philosophy of science, perhaps because it is taken for granted. One of its main treatments is in the form of 'epistemic iteration', a term devised by philosopher and historian of science Hasok Chang in his book *Inventing Temperature* [88]. He defines epistemic iteration as

> "A process in which successive steps of knowledge, each building on the preceding one, are created in order to enhance the achievement of certain epistemic goals. It differs crucially from mathematical iteration in that the latter is used to approach a correct answer that is known, or at least in principle knowable, by other means" [89].

Chang conceives epistemic iteration as 'corrective evolution', in which each step of understanding leads to others, but not straightforwardly:

> "What we have is a process in which we throw very imperfect ingredients together and manufacture something just a bit less imperfect" [90].

He points out how such a view of scientific process requires it to be defined pluralistically, according to a multiplicity of epistemic virtues and objectives of inquiry.

A few other philosophers of science have drawn attention to the iterative manner in which science develops. Iterativity particularly aids understanding of why imperfect models can serve as highly productive platforms for more complex, improved models [91,92]. We see this process happening again and again in synthetic biology, as false assumptions about the system are revealed in the construction of devices, thus leading to more effective devices and fuller understanding [23,93–95]. The much-celebrated biosynthesis of artemisinin precursors in microbial cells, for example, involved the piecemeal addition and re-engineering of genes and protein scaffolds to the original design in order to produce the right precursors in appropriate quantities [96]. Taking this same idea of 'false' starting points, Olaf Wolkenhauer suggests that activities such as systems biology, another successor science to genomics, can be defined as 'the art of making appropriate assumptions'. From his point of view,

> "the overwhelming complexity of cell-biological systems renders every attempt for comprehensive mathematical models futile. This does however not imply that we cannot improve our understanding of natural systems through mathematical modelling. Modelling in systems biology is a creative process by which different entailment structures are brought into congruence. The model is formulated to correspond in some useful way to observations made in experiments" [97].

Philosopher of science, Thomas Nickles, also discusses the way in which science manages to justify empirically 'some of its own starting assumptions' [98,99]. His work on how this happens emphasizes that scientific inquiry frequently depends on a 'multi-pass' progression, whereby researchers revisit and develop their problem-solving accounts and constructions of phenomena.

Scientists themselves are increasingly discussing the iterativity of research practices. Systems biology is an example of a field in which iterativity has become not only a virtue, but an aim and a guiding heuristic of inquiry. Practitioners in this field understand iteration as epistemic, as does Chang, but also methodologically. Iterativity is often characterized as a cycle of applications of methods that produces improved epistemic outcomes as the researcher moves from one phase of inquiry to the next

[43]. In some of these scientific accounts of iterativity, mathematical models are used as starting points for a repeated process of system perturbation and data integration. These interactions lead to more refined and accurate models, which are then subjected to further iterative inquiry [82,100–102]. Many outlines of the iterative methodology of systems biology include promiscuous combinations of discovery, description, experimentation, and hypothesis testing and generation [43,101,103,104].

Synthetic biology beautifully embodies the pragmatic nature of iterative scientific practice. Through iteration of exploratory, experimental, constructive and interpretive activities, synthetic biology's practitioners can generate useful knowledge, however imperfect their starting points [31,33,105,106]. 'It is a rare and joyous occasion when a synthetic genetic circuit actually works as expected the first time', confide Philippe Marguet et al. [107]. Through iterative activities such as design and construction, synthetic biologists build on and move beyond incomplete and poorly understood data [23,108]. Such iteration often includes further exploration of the phenomenal domain to see whether more knowledge can be gained [109]. This pragmatic pluralistic approach to the generation of scientific knowledge is exemplified by synthetic biologists of all stripes. Even the most ardent standardizers in the DNA-based device construction school admit they are willing to try a different, more eclectic approach 'if that works better' [110]: 'We'll have to figure it out. I don't know how to get to the right answer besides trying' [111]. But in the absence of thoroughgoing concrete evidence against it, the standardization approach has many potential practical reasons in its favour — at least for those who see the value and feasibility of making biology an engineering science. Whatever its future, standardization may function as one of the (false) starting points through which iterative practices produce improved understanding.

The initial successes of synthetic biology are due to the ongoing interplay of experimental, exploratory and construction-oriented strategies. While construction may be deliberately emphasized by synthetic biologists as the field's distinctive feature [38,107], the iterative interaction of techniques and approaches is characteristic of molecular biology generally over the last two decades. Iterative activities include general modes of practice, such as those I have described above as exploratory, but also include narrower and more precise lines of inquiry. Synthetic biology, even in its most rational, 'pure' engineering aspects, relies on iterative processes of design, construction, tinkering and improvement. But as a consequence of this 'corrective evolution' (to use Chang's term), synthetic biology and other molecular life sciences produce not idealized engineering objects but a bricolage of molecules, processes, technologies and knowledge.

4. Kludging

Synthetic biology is often described as a product of the engineering approaches that are entering biology as a response to parts lists provided by genomics and other high-throughput techniques [57]. In that process, engineering transforms not only techniques, but also the practice of data gathering and the research orientation of the field in which it arises. It is as engineers of biological systems that many synthetic biologists try to distinguish themselves from previous activities or fields such as genetic engineering, by claiming such activities are 'ad hoc' [112,113]. They argue that biology is finally able to overcome the irrationality of nature with human-made rational design [32,105]. Such design is usually taken to be the opposite of the kludge – a colloquial term for a workaround solution that is klumsy, lame, ugly, dumb, but good enough [114,115][1]. Kludging, contra rational design, emphasizes functional achievement, rather than the way in which that function is achieved. From a kludging perspective, it does not matter how inelegant the process, or how inefficient the relationships between the constructed parts. The ultimate vindication of construction is that the constructed system works.

Synthetic biology's design processes have always thus far involved iterative rounds of trial, error and pragmatic solutions, which are described as 'debugging', 'tweaking', or 'retrofitting' [23,33,86,95,107,116–118]. The reasons why such kludging needs to happen are at least threefold. First, the context-dependence of any designed part means that the uniformity and exact reproducibility of function — even in a redesigned and simplified system — cannot be expected [33,117,119]. Although synthetic biology's aim is to construct entire complex systems composed of standardized modules, at the moment, success is highly variable [13,31].

Second, biological systems are sub-optimal and complex products of evolution. Synthetic biology cannot simply replicate them through rational design processes [119,120]. Connections between designated modules are often unknown, and the complexity of evolved systems cannot be masked.

> "Combinations of well characterized biological parts to create synthetic wholes not only drives towards applications faster but also finesses past the underdetermination and crosstalking nonmodularity of natural systems. With the advent of facile synthesis and reusable modules, the evolutionary bricolage can be studied or avoided as needed" [121].

Consequently, many synthetic biologists see a pressing need to bypass evolutionary complexity [122].

Third, synthetic biologists also have to cope with the heterogeneity and noise of natural biological systems [63,65,123,124]. I have already mentioned the strides made through exploratory strategies in understanding biological noise. Noise sources include the fluctuation of transcription, translation and other biochemical processes within cells, which means there can be considerable phenotypic differences between genetically 'identical' cells

[1] There is a plurality of accounts of the origin and meaning of kludge (more common in the UK) and kluge (more often used in North America). The Wikipedia entry cited above combines most of these accounts and provides original references. An alternative backronym for kludge or kluge is, in fact, 'knowledge and learning used for good effect' is suggested by Koopman and Hoffman [115]. The relevance of this definition will become obvious as my discussion progresses.

in supposedly identical environments. Because of the inherent stochasticity in cellular processes, it is increasingly recognized that when different parts with known functions are combined into a system, this combination may produce unpredicted and completely novel capacities and behaviours [31,125].

All of this heterogeneity and evolutionary innovation has consequences for the type of engineering that can be done in synthetic biology. Diverse sources of variability obstruct synthetic biologists from achieving the desired 'plug and play' of predictable properties [126]. Even when it works, rational design requires multiple iterations of reconstruction and redesign [107,127]. Combinatorial synthesis and directed evolution — both employing 'irrational' biological processes to improve the functioning of designed devices — are increasingly necessary complements to or even replacements of rational design [93,95,123,128,129].

However, these partly randomized design processes produce constructions that are much more akin to bricolage. The constructions are achieved through tinkering and not pure rational engineering [113,130]. Rather than exemplifying rational, elegant and efficient design, many devices work because they are kludges.

> "unlike other engineering disciplines, synthetic biology has not developed to the point where there are scalable and reliable approaches to finding solutions. Instead, the emerging applications are most often kludges that work, but only as individual special cases. They are solutions selected for being fast and cheap and, as a result they are only somewhat in control" [131].

For example, the big synthetic biology success story of artemisinin production in microbial cells is based on cobbling together modified genes from different sources, with stabilization provided by engineered protein scaffolds, all pieced together in different constructs through a variety of methods [96,132–134]. This is not just the case for the engineering of biological systems, of course. Kludging of various sorts goes on constantly in electronic and software engineering. One such practice is the 'debugging' of software to make it work more effectively. Some software engineers use 'adaptation' to describe the process of how a kludge fits, augments and works around the constraints and shortcomings of systems and their operating environments [115].

Kludging, therefore, should not be interpreted as a failure of synthetic biology, but as a highly creative and effective process. Not only does kludging make things work, often in the context of non-standardized parts and insufficient knowledge, but it forms a conceptual nexus between biology, engineering and evolution. Organisms are sometimes discussed as 'clever hacks' that are the ad hoc products of multiple tinkering efforts [135]. Stephen Jay Gould believed that kludges, which he termed exaptations, ratcheted up with the increasing intricacy of the organism [136,137]. From this perspective, life should be understood as

> 'a collection of kludges taped together by chance and filtered by selection for functionality... It's the antithesis of planning and design — it's ad hoc co-option and opportunistic incorporation of chance enhancements. It's evolution' [138].

The metaphor of kludging can also be applied to scientific practice in biology, at least as a supplement to the over-idealized representation of experiment as a designed, efficient and linear inquiry, conducted by narrowing a research question into a refined hypothesis that obtains a specific answer. Some of the philosophers who discuss iterativity, and why science works despite starting from false assumptions, also mention the kludging that goes on in biological and other sciences [91,92,139,140]. Thomas Nickles describes this very aptly (although he does not call it kludging, but 'multi-pass inquiry' and bootstrapping):

> "Instead of constructing and searching the space of all possibilities for the optimal solution... we work out a tentative solution of questionable rigor, but one that 'works'!" [141,85].

If scientific experimentation is understood as kludging, then activities such as 'ad hoc' hypothesis modification cannot be rejected solely because they deviate from the linear path to knowledge – as has been the thrust of some philosophy of science [40]. The activities of constructing and modifying auxiliary interconnected models are inevitable aspects of scientific practice [142]. Especially (but not only) when grappling with multiple large data sets, creative efforts to piece them together are more likely to produce powerful results than will testing a single prediction [143]. I suggest it is useful to think of this as kludge-like epistemology.

The notion of kludging is reinforced by Max Delbrück's 'principle of limited sloppiness'. Delbrück used this term to describe the importance of not being excessively rigorous or controlled in experimentation [144]. He thought that too much precision would prevent novel insights, and that these might arise more readily if the researcher was flexible and responsive to the system of study and its variability. He used historical examples, such as experiments on the photoreactivation of bacteria and phage, to support his proposal [144] (for additional cases, see [145–147]). Experimental kludging, epistemic sloppiness and model 'fudging' do not make biologists inferior to engineers, however, because many sorts of engineers kludge to make things work. The proclivity for kludging may be deeply rooted because of how the mind itself has evolved as a kludge [148–150]. In scientific practice in general, and synthetic biology in particular, the emphasis on making things work drives kludging and its persistence.

While kludging may not describe every aspect of scientific practice, it is worthwhile at least to consider how it may be important for a pragmatic approach to knowledge and construction. This general claim needs to be understood, however, in relation to the fact that synthetic biology is in many respects anti-kludge: it wants nature and engineering to be elegant and efficient [151,11]. Kludging is a by-product, rather than an aim, whether it happens in science or evolution, so it cannot be offered as a normative account of science. Understanding kludging cannot therefore guide practice except in the sense of

suggesting that purely rational design is unlikely to work. Kludging does, however, function similarly to the way the notion of hypothesis testing does, but inversely. Whereas hypothesis testing is upheld as a rarefied ideal of science, kludging is discussed as the non-ideal, the thing to avoid. The best exemplification of this is to be found in the literature surrounding synthetic biology.

5. Conclusion

While I have used synthetic biology as my main exemplar, many other biological fields and areas of practice could probably be profitably analysed through these concepts of exploration, iterativity and kludging. The sketch I have made raises a number of questions that could fill in this outline and deepen our understanding of scientific processes of inquiry. One example is the tension between the constant build-up of kludges (experimental, synthetic and theoretical) and the necessity of simplification for cognitive and practical purposes [152]. It will be interesting to watch the development of synthetic biology to see if it manages to vanquish the former with the latter, and whether evolutionary understanding influences engineering practice (rather than the one-way application of engineering to biology, as happens now). Closer attention to iterativity, especially the relationships between its epistemic and methodological aspects, could also produce a richer picture of scientific practice. For example, it would be useful to gain insights into whether epistemic iterativity requires methodological iterativity, or whether single methods concerned narrowly with hypothesis testing can produce the same process of corrective evolution that emerges with iterative methodologies. The limits of exploratory investigation are another important issue on which to gain more insight. Discussions of exploratory modes of scientific practice need to examine whether they always encompass HD activities or whether they sometimes work alongside but independently of them. And a great deal more work could be done to find out which fields or cases of scientific practice are not covered by exploratory strategies.

Thinking about fields such as synthetic biology in light of these three aspects of scientific practice seems to indicate that the pursuit and enrichment of this framework could be worthwhile. Philosophy of science, by making an effort to understand multiple modes of investigation, their repeated interactions and the propensity to kludge, may be able to develop more extensive interpretive schema of scientific practice.

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References

[1] H.W. de Regt, S. Leonelli, K. Eigner, Scientific understanding: philosophical perspectives, University of Pittsburgh Press, Pittsburgh, 2009
[2] A.N.H. Creager, E. Lunbeck, M.N. Wise, Science without laws: model systems, cases, exemplary narratives, Duke University Press, Durham NC, 2007.
[3] H. Radder, The philosophy of scientific experimentation, University of Pittsburgh Press, Pittsburgh, 2003.
[4] T. Nickles, Configurations 6.1 (1998) 51.
[5] J. Griesemer, Am. Biol. Teach 47 (1985) 211.
[6] M.A. O'Malley, A. Powell, J.F. Davies, J. Calvert, BioEssays 30 (2008) 57.
[7] D. Endy, Nature 438 (2005) 449.
[8] T. Knight, Idempotent vector design for standard assembly of biobricks. MIT Synthetic Biology Working Group (2003), http://dspace.mit.edu/handle/1721.1/21168.
[9] http://parts.mit.edu.
[10] D. Ferber, Science 303 (2004) 158.
[11] J.D. Keasling, ACS Chem. Biol 3 (2008) 64.
[12] N.J. Guido, X. Wang, D. Adalsteinsson, D. McMillen, J. Hasty, C.R. Cantor, T.C. Elston, J.J. Collins, Nature 439 (2006) 856.
[13] C.A. Voigt, Curr. Opin. Biotechnol 17 (2006) 548.
[14] C. Lartigue, J.I. Glass, N. Alperovich, R. Pieper, P.P. Parmar, C.A. Hutchinson III, H.O. Smith, J.C. Venter, Science 317 (2007) 632.
[15] C. Lartigue, S. Vashee, M.A. Algire, R.-Y. Chuang, G.A. Benders, L. Ma, V.N. Noskov, E.A. Denisova, D.G. Gibson, N. Assad-Garcia, et al. Science 325 (2009) 1693.
[16] R. Gil, F.J. Silva, J. Peretó, A. Moya, Microbiol. Mol. Biol. Rev 68 (2004) 518.
[17] J.I. Glass, N. Assad-Garcia, N. Alperovich, S. Yooseph, M.R. Lewis, M. Maruf, C.A. Hutchinson III, H.O. Smith, J.C. Venter, Proc. Natl. Acad. Sci. U S A 103 (2006) 425.
[18] H.O. Smith, C.A. Hutchinson III, C. Pfanndoch, J.C. Venter, Proc. Natl. Acad. Sci. U S A 100 (2003) 15440.
[19] J. Cello, A.V. Paul, E. Wimmer, Science 297 (2002) 1016.
[20] L.Y. Chan, S. Kosuri, D. Endy, Mol. Syst. Biol. 1:2005.0018. (2005), doi:10.1038/msb4100025.
[21] G. Pósfai, G. Plunkett III, T. Fehér, D. Frisch, G.M. Keil, K. Umenhoffer, V. Kolisnychenko, B. Stahl, S.S. Sharma, M. de Arruda, V. Burland, S.W. Harcum, F.R. Blattner, Proc. Natl. Acad. Sci. U S A 312 (2006) 1044.
[22] E. Leonard, D. Nielsen, K. Solomon, K.J. Prather, Trends Biotechnol 26 (2008) 674.
[23] M. Heinemann, S. Panke, Bioinformatics 22 (2006) 2790.
[24] P.L. Luisi, F. Ferri, P. Stano, Naturwissenschaften 93 (2006) 1.
[25] R.V. Solé, A. Munteanu, C. Rodríguez-Caso, J. Macia, Philos. Trans. R. Soc. Lond., B, Biol. Sci 362 (2007) 1727.
[26] D. Deamer, Trends Biotechnol 23 (2005) 336.
[27] J.W. Szostak, D.P. Bartel, P.L. Luisi, Nature 409 (2001) 387.
[28] V. Noireaux, R. Bar-Ziv, J. Godefroy, H. Salman, A. Libchaber, Phys. Biol 2 (2005) 1.
[29] A.C. Forster, G.M. Church, Mol. Syst. Biol 2 (45) (2006), doi:10.1038/msb4100090.
[30] A. Moya, R. Gil, A. Latorre, J. Peretó, M.P. Garcillán-Barcia, FEMS Microbiol. Rev 33 (2008) 225.
[31] P.E.M. Purnick, R. Weiss, Nat. Rev. Mol. Cell Biol 10 (2009) 410.
[32] NEST New and Emerging Science and Technology, European Community, Synthetic Biology: Applying Engineering to Biology, European Commission Directorate General for Research, Brussels, (2005), http://www.univ-poitiers.fr/recherche/documents/pcrdt7/syntheticbiology.pdf.
[33] E. Andrianantoandro, S. Basu, D.K. Karig, R. Weiss, Mol. Syst. Biol. 2: 2006.0028 (2006), doi:10.1038/msb4100073.
[34] Keasling 2008(11), 65.
[35] A. Becskei, L. Serrano, Nature 405 (2000) 590.
[36] T.S. Gardner, C.R. Cantor, J.J. Collins, Nature 403 (2000) 339.
[37] M.B. Elowitz, S. Leibler, Nature 403 (2000) 335.
[38] D.A. Drubin, J.C. Way, P.A. Silver, Genes Dev 21 (2007) 242.
[39] F.J. Issacs, D.J. Dwyer, J.J. Collins, Nat. Biotechnol 24 (2006) 545.
[40] K.R. Popper, Conjectures and refutations: the growth of scientific knowledge, Routledge, London, 1963.
[41] J.F. Allen, BioEssays 23 (2001) 104.
[42] L. Laudan, Science and hypothesis: historical essays on scientific methodology, D. Reidel, Dordrecht, 1981.
[43] D.B. Kell, S.G. Oliver, BioEssays 26 (2004) 99.
[44] D.J. Glass, N. Hall, Cell 134 (2008) 378.
[45] W.S. Harwood, J. Coll. Sci. Teach 33 (2004) 29.

[46] E.F. Caldin, HYLE – Int, J. Phil. Chem. 8 (2002) 103.
[47] D. Allchin, PSA 1 (1992), p.74.
[48] M.A. Oberhardt, B.Ø. Palsson, J.A. Papin, Mol. Sys. Biol 320 (5) (2009), doi:10.1038/msb.2009.77.
[49] C. Wilson, The Invisible world: early modern philosophy and the invention of the microscope, Princeton University Press, Princeton, 1995.
[50] M.J. Ratcliff, The quest for the invisible: microscopy in the enlightenment, Ashgate, Farnham, Surrey, 2009.
[51] J. Farley, The spontaneous generation controversy from Descartes to Oparin, Johns Hopkins University Press, Baltimore, 1974.
[52] F.J. Ayala, Proc. Natl. Acad. Sci. U S A 106 (Suppl. 1) (2009) 10033.
[53] M. Ghiselin, The triumph of the Darwinian Method, University of California Press, Berkeley, 1969.
[54] D. Penny, Trends Evol. Biol e1 (1) (2009), doi:10.4081/eb.2009.e1.
[55] M.J.S. Hodge, in : D.S. Bendall (Ed.), Evolution from molecules to men, Cambridge University Press, Cambridge, 1983, p. 43.
[56] T. Lewens, Darwin, Routledge, London, 2007.
[57] R. Brent, Cell 100 (2000) 169.
[58] R. Aebersold, L.E. Hood, J.D. Watts, Nat. Biotechnol 18 (2000) 359.
[59] L. Franklin, Phil Sci 72 (2005) 888.
[60] B. Strasser, Science 322 (2008) 537.
[61] S.A. Benner, A.M. Sismour, Nat. Rev. Genet 6 (2005) 533.
[62] P.S. Swain, M.B. Elowitz, E.D. Siggia, Proc. Natl. Acad. Sci. U S A 99 (2002) 12795.
[63] M.B. Elowitz, A.J. Levine, E.D. Siggia, P.S. Swain, Science 297 (2002) 1183.
[64] W.J. Blake, M. Kaern, C.R. Cantor, J.J. Collins, Nature 422 (2003) 633.
[65] A. Raj, A. van Oudenaarden, Cell 135 (2008) 216.
[66] N. Rosenfeld, J.W. Young, U. Alon, P.S. Swain, M.B. Elowitz, Science 307 (2005) (1962).
[67] A. Warmflash, A.R. Dinner, Proc. Nat. Acad. Sci. U S A 105 (2008) 17262.
[68] B. Munsky, B. Trinh, M. Khammas, Mol. Sys. Biol 318 (5) (2009), doi:10.1038/msb.2009.75.
[69] J. Hasty, J. Pradines, M. Dolnik, J.J. Collins, Proc. Nat. Acad. Sci. U S A 97 (2000) 2075.
[70] J.C.W. Locke, M.B. Elowitz, Nat. Rev. Microbiol 7 (2009) 383.
[71] A. Eldar, V.K. Chary, P. Xenopoulos, M.E. Fonts, O.C. Losón, J. Dworkin, P.J. Piggot, M.B. Elowitz, Nature 460 (2009) 510.
[72] T. Çağatay, M. Turcotte, M.B. Elowitz, J. Garcia-Ojalvo, G.M. Süel, Cell 139 (2009) 512.
[73] J. Hasty, D. McMillen, J.J. Collins, Nature 420 (2002) 224.
[74] A. Loettgers, Biol. Theory 2 (2007) 134.
[75] A. Loettgers, Biol. Theory 4 (2009) 340.
[76] A.C. Love, International Society for the History, Philosophy, and Social Studies of Biology, Brisbane (2009), http://www.ishpssb2009.org/program.html.
[77] R.M. Burian, Hist. Philos. Life Sci 19 (1997) 27.
[78] F. Steinle, Phil. Sci. 64 Suppl. (1997) S65.
[79] K. Elliott, Hist. Philos. Life Sci 29 (2007) 313.
[80] M.A. O'Malley, Hist. Philos. Life Sci 29 (2007) 337.
[81] D.J. Glass, Experimental design for biologists, Cold Spring Harbor Laboratory Press, CSH, 2006.
[82] H. Kitano, Nature 420 (2002) 206.
[83] M.A. O'Malley, K.C. Elliott, C. Haufe, R.M. Burian, Cell 138 (2009) 611.
[84] http://undsci.berkeley.edu/article/0_0_0/howscienceworks_02.
[85] T. Nickles, Synthese 253 (69) (1986) 260.
[86] T. Ellis, X. Wang, J.J. Collins, Nat. Biotechnol 27 (2009) 465.
[87] E. Cule (2009), http://network.nature.com/people/erikacule/blog/2008/11/11/whats-up-down-fast-robust-and-tunable (accessed November 7th, 2009).
[88] H. Chang, Inventing temperature: measurement and scientific progress, Oxford University Press, Oxford, 2004.
[89] H. Chang, 88(2004)p. 253.
[90] H. Chang, 88(2004) p. 226.
[91] W. Wimsatt, in : M. Nitecki, A. Hoffman (Eds.), Neutral models in biology, Oxford University Press, New York, 1987, p. 23.
[92] W.C. Wimsatt, Re-engineering philosophy for limited beings: piecewise approximations to reality, Harvard University Press, Cambridge MA, 2007.
[93] T. Koide, W.L. Pang, N.S. Baliga, Nat. Rev. Microbiol 7 (2009) 297.
[94] J. Stricker, S. Coodson, M.R. Bennett, W.H. Mather, L.S. Tsimring, J. Hasty, Nature 456 (2008) 516.
[95] E.L. Haseltine, F.H. Arnold, Ann. Rev. Biophys. Biomol. Struct 36 (2007) 1.
[96] K.L.J. Prather, C.H. Martin, Curr. Opin. Biotechnol 19 (2008) 468.
[97] O. Wolkenhauer, Emerging Methodologies in Mathematical Modelling in Biology and Medicine, Edinburgh (2009), http://www.icms.org.uk/workshops/modellingmethodologies.
[98] T. Nickles, Biol. Phil. (12) (1997) 127–133.
[99] T. Nickles, Danish Yearbook of Philosophy 32 (1997) 11.
[100] A. Aderem, Cell 121 (2005) 511.
[101] C. Auffray, S. Imbeaud, M. Roux-Rouquié, L. Hood, C. R. Biol 326 (2003) 879.
[102] F.J. Bruggeman, H.V. Westerhoff, Trends Microbiol 15 (2006) 45.
[103] Systems Biology Institute, Methodologies of systems biology, (2008), http://www.systemsbiology.org/Systems_Biology_in_Depth/Methodologies_of_Systems_Biology.
[104] T. Ideker, T. Galitski, L. Hood, Annu. Rev. Genomics Hum. Genet 2 (2001) 343.
[105] P.M. Boyle, P.A. Silver, J. R. Soc. Interface 6 (Suppl. 4) (2009) S535.
[106] Y. Matusoka, S. Ghosh, H. Kitano, J. R. Soc. Interface 6 (Suppl. 4) (2009) S393.
[107] P. Marguet, F. Balagadde, C. Tan, L. You, J. R. Soc. Interface 4 (2007) 607–614.
[108] B. Canton, A. Labno, D. Endy, Nat. Biotechnol 26 (2008) 787.
[109] L. Endler, N. Rodriguez, N. Juty, V. Chelliah, C. Laibe, C. Li, N. Le Novère, J. R. Soc. Interface 6 (Suppl. 4) (2009) S405.
[110] Endy, personal communication, 2009.
[111] D. Endy, Ind. Biotechnol 4 (2008) 340–349.
[112] R. Brent, Nat. Biotechnology 22 (2004) 1211.
[113] A.P. Arkin, D.A. Fletcher, Genome Biol 114 (7) (2006), doi:10.1186/gb-2006-7-8-114.
[114] http://en.wikipedia.org/wiki/Kludge.
[115] P. Koopman, R.R. Hoffman, IEEE Intell. Sys (2003) 70.
[116] M.A. O'Malley, Biol. Theory 4 (2009) 378.
[117] L. Serrano, Mol. Syst. Biol 158 (3) (2007), doi:10.1038/msb4100202.
[118] C.L. Barrett, T.Y. Kim, H.U. Kim, B.Ø. Palsson, S.Y. Lee, Curr. Opin. Biotechnol 17 (2006) 1.
[119] A. Arkin, Nat. Biotechnol 26 (2008) 771.
[120] D. Sprinzak, M.B. Elowitz, Nature 438 (2005) 443.
[121] G.M. Church, Mol. Syst. Biol. (2005), doi:10.1038/msb4100007 p. 2.
[122] C. Hold, S. Panke, J. R. Soc. Interface 6 (Suppl. 4) (2009) S507.
[123] W.J. Blake, F.J. Issacs, Trends Biotechnol 22 (2004) 321.
[124] J. Paulsson, Nature 427 (2004) 415.
[125] M.L. Simpson, Trends Biotechnol 22 (2004) 555.
[126] H. Koyabashi, M. Kærn, M. Araki, K. Chung, T.S. Gardner, C.R. Cantro, J.J. Collins, Proc. Natl. Acad. Sci. U S A 101 (2004) 8414.
[127] K. Michalodimitrakis, M. Isalan, FEMS Microbiol. Rev 33 (2008) 27.
[128] C.C. Guet, M.B. Elowitz, W. Hsing, S. Leibler, Science 296 (2002) 1466.
[129] Y. Yokobayashi, R. Weiss, F.H. Arnold, Proc. Natl. Acad. Sci. U S A 99 (2002) 16587.
[130] T.K. Lu, A.S. Khalil, J.J. Collins, Nature Biotechnol 27 (2009) 1139.
[131] Arkin and Fletcher. 114(2006) p. 4.
[132] V.G. Yadav, G. Stephanopoulos, Curr. Opin. Microbiol 13 (2010) 371.
[133] D. Na, T.Y. Kim, S.Y. Lee, Curr. Opin. Microbiol 13 (2010) 363.
[134] J.E. Dueber, G.C. Wu, G.R. Malmirchegini, T.S. Moon, C.J. Petzold, A.V. Ullal, K.L.J. Prather, J.D. Keasling, Nature Biotechnol 27 (2009) 753.
[135] S. Huang, J. Wikswo, Rev. Physiol. Biochem. Pharmacol 157 (2006) 81.
[136] S.J. Gould, Proc. Natl. Acad. Sci. U S A 94 (1997) 10750.
[137] S.J. Gould, E.S. Vrba, Paleobiol 8 (1982) 4.
[138] P.Z. Myers, (2008), http://seedmagazine.com/content/article/algorithmic_inelegance/.
[139] W Goodwin, Paper presented at models and simulations 3, Charlottesville, Virginia (2009), http://philsci-archive.pitt.edu/archive/00004517/.
[140] J. Lenhard, E. Winsberg, (2009), http://www.cas.usf.edu/~ewinsb/papers.html.
[141] T. Nickles, 94(1997)p. 137.
[142] I. Lakatos, Proc. Aristotelian Soc. 69(1968–1969)p. 149.
[143] T.R. Gregory, (2009), http://network.nature.com/people/trgregory/blog/2009/03/21/flaws-of-the-fudge-factor.
[144] M. Delbrück, Oral History Project, California Institute of Technology Archives, CA (1979), http://oralhistories.library.caltech.edu/16/ p. 76–77.
[145] R.S. Root-Bernstein, Perspect. Biol. Med 32 (1989) 472.
[146] Y.N. Jan, L.Y. Jan, Int. J. Dev. Biol 42 (1998) 531.
[147] F. Grinnell, FASEB J 23 (2009) 7.
[148] A. Clark, Mind Lang 2 (1987) 277.
[149] G. Marcus, Kluge:, The haphazard evolution of the human mind, Houghton Mifflin, NY, 2008.
[150] D.J. Linden, The accidental mind: how brain evolution has given us love, memory, dreams, and god, Harvard University Press, Cambridge, MA, 2007.
[151] D. Endy, The Economist (2006), http://www.economist.com/science/displaystory.cfm?story_id=7854314.
[152] U. Alon, Nature 446 (2007) 497.