Synthetic Biology and the Search for Alternative Genetic Systems: Taking How-Possibly Models Seriously

Rami Koskinen, *University of Helsinki*

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**Abstract**

How-possibly models are usually treated as some kind of second-rate theoretical tools. They may be indispensable in the early stages of theorizing, but do not constitute the main aim of modeling, namely, the discovering of a one true mechanism responsible for the phenomenon under study. I argue that this prevailing picture does not do justice to the synthetic strategy that is commonly used in the engineering sciences. In synthetic biology, how-possibly models are not something to be eliminated by a more detailed analysis, but rather design hypotheses for a field whose ulti­mate goal is to build novel biological systems.

**1. Introduction**

According to the most influential contemporary account of explanation in the philosophy of biology, namely, that of mechanistic explanation, the aim of the life sciences is seen as the discovering and modeling of mechanisms that “produce, underlie, or maintain a phenomenon” that is being studied (Craver and Darden 2013, 15). The mechanistic strategy of modeling is typically conceptualized as proceeding by somehow constraining a space of possible mechanisms for a given phenomenon or function. According to Craver (2007, 31), the space of possible mechanisms contains all the mechanisms that could possibly explain a phenomenon. By explicating a particular point in this space, scientists construct a *how-possibly explanation* or *model*. Furthermore, it is often assumed that there is just one true or correct mechanism, the details of which are ideally captured in a finished *how-actually* *model*. Intermediate between these two extremes are *how-plausibly models*, which form a more tightly constrained subset of how-possibly models, but still lack the full empirical support of a how-actually model. (Craver and Darden 2013, 34–35.)

According to the prevailing picture of biological modeling strategy, a successful search for a mechanistic explanation should converge on one single mechanistic model candidate, and divergent how-possibly models that differ in their mechanistic details should be discarded as superfluous and scientifically incorrect. According to Craver (2007, 131), “Distinguishing good explanations from bad requires that one distinguishes real components from fictional posits. The most dramatic examples of fictional posits include animal spirits, entelechies, and souls, but fictitious entities can be far more mundane than these”. He concludes that many how-possibly mechanisms “require parts (and activities) that *do not exist*” (Craver 2007, 131, my emphasis). Because modern-day scientists want their models to work, and in particular, do not want to commit themselves to any kind of spooky non-existent entities, how-possibly models are usually considered as something that should be eliminated as quickly as possible when conducting serious research. In contemporary philosophy of science, how-possibly models are often treated as some kind of second-rate explanations or theoretical tools (e.g., Rosenberg 2006, 45; see also Craver 2006, 361, 2007, 112).

In this paper I will argue that this current view concerning the role of how-possibly models is very narrow. More precisely, it may be a good approximation in the context of scientific analysis of natural systems where research advances through the methods of decomposition and localization (Bechtel and Richardson 1993/2010). However, this does not preempt all the goals of biological investigation. The idea of starting from a range of possible models and then working towards one or a very limited number of how-actually models seems to make much sense when one considers the general purpose of biological investigation. For example, given that one of the main aims of science is to provide manageable generalizations that unify phenomena as much as possible, focusing on how things actually work is a neat idea and surely a good starting point! Especially, it is much more manageable to model complex high level input­–output phenomena when they can be cashed out in terms of a few select mechanisms that are already familiar. Another reason that is especially prominent in the life sciences is the ability to effectively intervene on various target systems for medical purposes (see Craver 2007). Why would scientists bother wasting their time with mere how-possibly models that do not provide good access to actual phenomena, not to mention ways to effectively intervene on them?

However, although it is often the case that scientists are interested in some well-defined actual target system, it is also true that a lot of times the target of investigation is some more abstract feature of the living world that might require studying objects that, strictly speaking, do not exist, at least at the moment of investigation (Dawkins 1986; Dennett 1995, 102–103). I hold that the same is true also in the context of the synthetic strategy that is commonly used in the engineering sciences. In the field of synthetic biology, researchers use how-possibly models to study what may be called potential biological systems. I argue that in the hands of bioengineers, abstract how-possibly models are not something to be eliminated by a more detailed analysis, but rather design hypotheses for a field whose ulti­mate goal is to build novel biological systems and “re-wire” existing ones. I explicate this role further by providing an example from the study of alternative genetic systems by synthetic biologist Steven Benner and his group. The case will highlight how the method of synthesis, even when it fails, provides an effec­tive way to limit the space of possible models for biological mechanisms. This has effects for the study of potential and actual natural systems alike.

**2. From Actual to Potential Biological Systems**

It is often said that one important thing about mechanistic understanding is the ability to answer “what-if-things-had-been-different” questions (e.g., Craver 2006, 358, following Woodward 2003). This is certainly true in the sense that, ideally, when a mechanism is fully understood (i.e., our best model of it does not contain any black boxes left to open) we are able to reliably predict its output for a range of input and parameter values and even manipulate its functioning. Knowing how an actual mechanism operates as accurately as possible gives us more effective ways to handle typical contrafactual questions that arise in science (cf. Craver 2006). However, this kind of access to full mechanistic details of actual target systems forms only one part of contrafactual reasoning that is of interest to scientists. Sometimes, especially when dealing with some more theoretical issues, scientists who ask “what-if-things-had-been-different” questions are not in fact inquiring how accurately we understand the parts and workings of some actual mechanism. Rather, I suggest, they might be wondering whether the mechanism (or the system in general) itself could, or could have been, different. It is in this way that, instead of being just an eliminable scaffold on the way towards a how-actually model, a how-possibly model can become the main object of inquiry in its own right.

Taking how-possibly models of biological systems seriously in the above sense might mean two things. First, it might simply mean taking seriously the general strategy of “turning the tables around”, that is, focusing research on what is possible instead of actual in the biological world. This is akin to an exploration into the dark where rather few things limit the search space. Second, it might mean that one is committed to the study of some particular how-possibly model or set of models for a phenomenon for which there already is a how-actually model. The second version has the nice advantage that we already have an existential proof that *that* phenomenon or function is indeed realizable at all. We can then investigate whether it can be achieved by means of some alternative mechanism; the strategy is essentially *contrastive* in nature. Indeed, this is something that is done in many quarters of biological engineering and especially in the field of synthetic biology.

As in the case of more traditional life scientific research, mechanistic understanding and modeling of biological systems is at the heart of synthetic biology. In a sense, synthetic biology can be seen as taking them even further. The field is often characterized by a strive to build novel biological systems (Elowitz and Lim 2010). Because this requires an excessive ability to manipulate existing biological mechanisms, synthetic biology is often portrayed as the ultimate test for our mechanistic understanding of the living world in general (Endy 2005; Elowitz and Lim 2010).[[1]](#footnote-1) However, at the same time these bioengineers are also testing completely new waters by expanding biological understanding over and above naturally occurring systems. Some of the synthetic systems, like artificial genetic circuits, that have been built can be seen both as new biological objects in their own right and as certain kind of concrete, but theoretical models of what kind of design principles are biologically feasible (Knuuttila and Loettgers 2013). Although the study of these potential biological systems is targeted at certain very specific types of how-possibly models of biological systems, they can be seen as enriching our understanding of biology in terms that go beyond mere engineering feats (Morange 2009).

For example, it has been suggested that synthetic design methods might be able to prove valuable information about the nature and limits of the evolution of gene regulation:

[An important problem in evolutionary biology is] why the genetic network architectures we observe in Nature have evolved to solve a particular problem an organism faces in its environment. This challenge is often complemented by the question of which selective forces (i.e. environmental or cellular conditions) have shaped the biological systems we observe in modern organisms. The null hypothesis is simply that a particular architecture has arisen by non-selective forces and that multiple architectures would be sufficient to achieve the biological functionality observed. (Bayer 2010, R775.)

In normal evolutionary research, these kinds of questions are often difficult to evaluate because many specific functions are found only in a very limited number of systems or model organisms; the relevant sample might also be biased by historical contingency. Modeling the mechanistic details of the actual system in ever greater detail does not seem to be of much help here. However, thanks to synthetic biology and other forms of biological engineering, evolutionary studies are no more necessarily restricted by the availability of naturally occurring systems: “The construction of synthetic versions of natural circuits is a powerful way to interrogate questions of ‘why’ in biology” (Bayer 2010, R775). Endy also defines one of the main goals of synthetic biology as follows: “[…] synthetic biology provides an opportunity to test the hypothesis that the genomes encoding natural biological systems can be ‘re-written’, producing engineered surrogates that might usefully supplant some natural biological systems” (Endy 2005, 449; see also Sprinzak and Elowitz 2005).

The re-design strategy depicted here limits its focus on systems that differ in their underlying mechanistic architecture, but that are nevertheless capable of realizing the same higher level function. It is reminiscent of the situation that philosophers often call by the name “multiple realizability”. Because how-possibly models are often presented in exactly this kind of situation where they are in a sense explanatory rivals for one and the same phenomenon, it is easy to see how they fit into the conceptual scheme of “biological re-writers”. One of the most compelling examples of this kind of research comes from the study of artificial genetic systems that can be regarded as functional alternatives for our natural DNA. It is there that various how-possibly models, on top of their more traditional explanatory purport, seem to have the role of explicit design hypotheses.

**3. Alternative Alphabets for Life’s Code**

Why is the genetic code based on the DNA molecule? Is it a functional necessity, or just a historical accident? Because the sample size of life on Earth is one, there is no straightforward empirical way to investigate this issue. In his famous booklet *What is Life?* the physicist Erwin Schrödinger (1944) originally proposed an inspirational how-possibly model for genetic material in which genes were hypothesized as consisting of some kind of aperiodic crystals. This was nine years before Watson and Crick’s discovery of the structure of the DNA molecule. Because of their groundbreaking work, we, of course, now have an excellent how-actually model for the implementation of the genetic material. However, as successful as their model has turned out to be, it does not really answer all the why-questions that can be raised regarding the material nature of the genetic code. To answer these questions would require contrasting DNA with some other plausible how-possibly models for genetic material and hoping for some principled clue as to why nature has opted for this particular solution.

Beginning already in the late 1980’s (Switzer, Moroney, and Benner 1989), synthetic biologist and chemist Steven Benner has been studying what can be called artificial genetic systems. These are chemical structures that are supposed to have the essential functional features of a genetic code, but that are nevertheless different from the structural design of familiar DNA and RNA molecules. According to Benner:

In a version popular today in some engineering communities, [synthetic biology] seeks to use *natural* parts of biological systems (such as DNA fragments and protein “biobricks”) to create assemblies that do things that are *not* done by natural biology (such as digital computation or manufacture of a speciality chemical). […] Among chemists, “synthetic biology” means the opposite. Chemist’s “synthetic biology” seeks to use *unnatural* molecular parts to do things that are done by natural biology. Chemists believe that if they can reproduce biological behavior *without* making an exact molecular replica of a natural living system, then they have demonstrated an understanding of the intimate connection between molecular structure and biological behavior. If taken to its limit, this synthesis would provide a chemical understanding of life. (Benner, Yang, and Chen 2011, 372; emphasis in original.)

For the last 25 years Benner has done just that. That is, he has studied a wide range of different chemical systems that could potentially be used to fulfill the same role as DNA/RNA in naturally evolved organisms. Although no such system still exists, researchers have managed to construct many interesting variants that have at least some of the features required of a code of life, and new exciting results are frequently being reported from scientists working at the junction between synthetic biology and chemistry (see, e.g., Malyshev at al. 2014; Marlière et al. 2011; Thyer and Ellefson 2014).

Although these studies are limited and far from conclusive, they nevertheless provide reasons to believe that DNA might not be the kind of necessary ingredient that some take it to be as the only thing capable of turning inanimate matter into living, reproducing, and evolving systems. In the language of Craver and Darden (2013, 69), they give us good reasons to suppose that alternative genetic systems remain a *live possibility*. To make the continued hegemony of DNA as the biochemical medium of choice seem even less secure, Benner noted in an interview published in *Nature* in November 2012 that “The first thing you realize is that [DNA] is a stupid design”, further insisting that, “If you were a chemist setting out to design this thing, you would not do it this way at all.” (Kwok 2012, 516.)

However, it is one thing to criticize the structural design of DNA, and another to show that any other chemical solution would be able to perform the same functions. Because all known organisms have their genetic code based on DNA/RNA, the possibility of “alternative genetic alphabets” requires strong empirical proof (Thyer and Ellefson 2014, 291). One would expect there to be good chemical and evolutionary reasons for DNA to be the medium for genetic information. Although it is nowadays recognized that biological solutions are not always optimal in the strong sense, they are nevertheless often extremely robust and surprisingly efficient (see Wagner 2005). Because so much complex, evolutionarily successful life is based, at the bottom level, on the structural features of DNA, it simply cannot be *that* inadequate as a molecule. However, it is also because of this most intense of dependencies that it is actually very difficult to make many far reaching biological conclusions about the nature of DNA; it is a deeply generatively entrenched fact about the living world (Wimsatt 2007, 135–136).

To understand the requirements for an adequate genetic code, I will first have to examine the definition of a living system that Benner and others advocate. This is a working definition, which means that it is open for revisions. It nevertheless captures many of the features that biologists take to be essential for living systems. In his work, Benner follows the so-called “NASA definition” of life as a self-sustaining chemical system capable of Darwinian evolution (Benner, Yang, and Chen 2011, 375). Although this definition leaves many important facts up for further refinement, it nevertheless already makes some empirical bets by, for example, ruling out genuinely Lamarckian systems. As Benner himself notes, we do not have any reasons to believe that even Lamarckian systems would be strictly impossible (Benner, Yang, and Chen 2011, 375). However, we have to start from somewhere, and at least we have many empirical examples of Darwinian life, not to mention a particularly successful evolutionary framework that unifies these findings. In a sense, synthetic biologists can take the testing of this theory even further by trying to come up with some other kind of chemical systems that can be subsumed under it.

Benner’s abstract model of a genetic system has three features: (1) the ability to carry biological information, (2) the ability to transmit biological information, and (3) the ability to support Darwinian evolution (based on Benner, Yang, and Chen 2011). In practice, the above list can be thought to encompass also some implicit auxiliary assumptions à la Duhem and Quine. Examples of these could be some kind of linear arrangement of the code, or the overall chemical and thermodynamic stability of its structure (see Szathmáry 2003). These general features can also be broken down into smaller mechanisms or causal role functions that make them physically feasible. For example, the encoding of biological information is often taken to require some kind of chemical specificity, like bonding, lock-and-key complementarity, and so on. This brings the whole enterprise closer to the how-plausibly end of all conceivable possibilities.

Although the above list seems quite simple, it contains an implicit tension that makes it more difficult to achieve all of the requirements simultaneously. It is obvious that without the first requirement, we would simply have no code at all­­––genetic or other kind. However, the mere ability to store information is not that interesting property in itself. The code must also be able to transmit information from one system to another. It is only after this step is fulfilled that we can actually speak about inheritance. Taken in isolation, the requirements (1) and (2) suggest that the more accurate the functions in question are, the better the medium is in realizing the code. In a sense this is true. A system that transmits its information content so poorly that descendant systems hardly resemble their parent systems would clearly not be able to support Darwinian life. However, what the requirement (3) implicitly insists is that although the copying process should be reliable, it should not be completely certain. Otherwise no variation is ever going to accumulate, and the system can only produce an endless army of genetically identical clones. Again, the space of possible models for genetic material is in this way constrained before any considerations about the physical medium has taken place.

According to Benner, many synthetic biologists’ original expectation was that the best place to start changing the chemical basis of the genetic code was the sugar backbone of natural DNA molecules. This is because the informational specificity of the genetic code is often thought to lie in the highly specific complementary base pairings between the nucleobases A-T and C-G. The backbone was believed to be just a contingent structure, a kind of molecular “scaffold”, whose purpose is to support the real sources of information. (Benner, Yang, and Chen 2011, 376.) However, as it turned out after numerous trials, Benner and his team were unable to achieve functionally stable molecules by changing the sugar backbone. For example, backbones made of glycerol units turned out be too flexible and the whole structure broke down in normal temperatures; the nucleobase bindings alone were not strong enough to hold the structure together. (Benner, Yang, and Chen 2011, 377.)

In addition to sugars, the DNA backbone features also phosphates. Similar results were attained by Benner and his team when they tried to change the phosphates as did in the case of the sugars. For example, when the phosphates were replaced by synthetic oligosulfones, the structure tended to fold onto itself. (Benner, Yang, and Chen 2011, 378.) This was bad news, because folded structures might not be specific enough to ensure faithful pairing. Moreover, if the structure is stripped off of its repeating charges that are manifest in the phosphates, it might hamper its mutability; remember that the ability to evolve is one of the functional requirements of life that Benner advocates in his working model. (Benner, Yang, and Chen 2011, 379–380.) Thus, the sugar and phosphate backbone with its repeating charges seemed to be a necessary feature of a biologically plausible genetic system capable of Darwinian evolution.[[2]](#footnote-2)

This meant that in order to achieve the grand goal of an alternative genetic system, the changes must be made to the nucleobases. After trying out numerous working hypotheses or possible models for a genetic code that is based on alternative or “unnatural” alphabets, Benner and his team finally arrived at the six letter alphabet (A, T, C, G, P, Z). Using methods from modern biological engineering, like polymerase chain reaction, Benner and his team were able to add the bases P and Z to a system based on the natural (A, T, C, G) alphabet. These bases were selected because bonding between them has experimentally been shown to be very strong and specific. Furthermore, unlike in the case of alternative backbones, the new bases *can* support Darwinian evolution: In the case of the (A, T, C, G, P, Z) alphabet, the new bases have been shown to be mutable to natural bases. Also, and perhaps even more interestingly, C and G could mutate to give P and Z. (Benner, Yang and, Chen 2011, 384.)

Benner’s alphabet has some very interesting biological properties. First, it is a mix of both natural and unnatural biochemical, or genetic, “letters”. Second, its cardinality differs from that of the natural DNA/RNA alphabet. These two features make it possible to use the new alphabet to study both disparate *and* expanded genetic alphabets at the same time––a double win. I maintain that these features also make it an interesting case to study various how-possibly models of genetic systems.

Do the incorporated bases P and Z radically differ from the molecular structure of those of the natural nucleobases? This is somewhat debatable. It is true that the molecular mechanisms of pairing between them resemble those of A-T and C-G. However, they are still structurally different molecules. It was certainly far from clear that these bases could be inserted successfully into a system of natural alphabet. This is especially so because they tend to change the “dynamics” of the whole structure. The more different types of parts that can possibly interfere with each other there are, the more likely it is that the whole system will fail to be able to perform its functions properly; the basic parts of a mechanism often seriously constrain its space of possible models (cf. Craver and Darden 2013: 105). Also, the informational change that is brought by the new nucleobases can be as interesting as the change in structural features. With alphabets of different cardinality, it is possible to test the idea whether the functions of living systems can be coded in ways that differ from the familiar four-letter system. Although previous models had been susceptible about this (Szathmáry 2003), Benner’s (A, T, C, G, P, Z) alphabet is one of the first experimental results to give compelling reasons to believe that they do.

**4. Conclusions**

Contrary to traditional life-scientific practice, the engineering or “synthetic” strategy of fields like synthetic biology can be used to explore the space of biological possibilities by taking established how-actually models for biological systems as a starting point, and then working towards realizing alternative and contrasting how-possibly models. If it succeeds, it opens up new exciting possibilities. For example, a fully functional alternative genetic alphabet could work as a genetic “firewall” between engineered and naturally evolved organisms, providing an effective biosafety tool (Schmidt 2010). However, even if it does not, something new is still being learned about the nature of actual systems. Both situations can have benefits for basic science.

Besides Benner’s group, many others have also been working on synthetic genetic systems and alternative alphabets. For example, Marlière et al. (2011) report a successful incorporation of a new nucleobase 5-chlorouracil into a laboratory strain of *E. coli*, while Malyshev et al. (2014) produced similar experiments with the pair d5SICSTP-dNaMTP. What is remarkable is that both cases exemplified robust functionality with no obvious biological pitfalls. It might be that some of the structural features of DNA, like the repeating charges along its backbone, are essential; so-called forced moves in the space of available design (see Dennett 1995, 128–131). However, with the case of the familiar genetic alphabet, it seems that nature *could have* chosen otherwise, but for some reason it simply did not. It seems to be a partly contingent solution. Because it is not possible for evolution to change this situation anymore, the only plausible way to study these questions is to use synthetic design methods. This is also good way to naturalize the notion of biological possibility: To show that how-possibly models and speculative scenarios of evolutionary theory and the rest of biology can be given a philosophically satisfying reading.

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1. Craver and Darden (2013, 92–94) also mention the importance of engineering or “build it” test as an effective way to further refine scientific understanding of biological mechanisms. [↑](#footnote-ref-1)
2. In the case of some *xeno nucleic acids*, researchers have been able to change the sugar backbone of DNA molecules. However, it is not clear whether these systems can support life. See Schmidt (2010). [↑](#footnote-ref-2)