

Soft to Wet: Morphogenetic Engineering in Synthetic Biology

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Toward a Biological Compiler

Synthetic biology is an emerging scientific discipline that promotes the standardized manufacturing of biological components without natural equivalents. It is currently in search of design principles to achieve a reliable and secure level of functionality from reusable biological parts, as exemplified by BioBricks. The goal is to create artificial living systems that can meet various needs in application domains such as health care, nanotechnology, energy, and chemistry. So far, most of the studies in this field have focused on the low level, seeking to characterize and validate the elementary properties of an individual bacterium. However, beyond genetic engineering problems and bioinformatics tools, computer scientists also view synthetic biology as a *systems design* challenge, and liken it to large software systems and electronic circuits.

In this context, the SynBioTIC project (Delaplace et al., 2010) is positioned upstream, at the *cell population level*—assuming that the necessary low-level control mechanisms are already in place. From the “wetware” viewpoint, its motivation is to exploit of the nontrivial *collective properties* of bacteria. To this aim, SynBioTIC proposes to design and develop formalisms and computer tools to literally “compile” (as in programming languages) the overall behavior of a population of cells into processes local to each cell. It relies on the specification of a global spatial behavior and its description across a *tower of languages*. Each language at a given level addresses distinct features. Its set of instructions can be compiled into the lower level, and ultimately down to the final bioware into a cellular regulation network (gene network, signaling and metabolic pathways). This “soft-to-wet” approach, similar to a classical “soft-to-hard” compiler, aims to fill the gap between the high-level description of a biosystem and its low-level physical requirements.

From the software viewpoint, this long-term core research project also belongs to the “unconventional/natural computing” family (Amos et al., 2012), which promotes non-Turing, *in materio* architectures at the interface between computer science and biological engineering. It relies on the development of new approaches such as spatially ex-

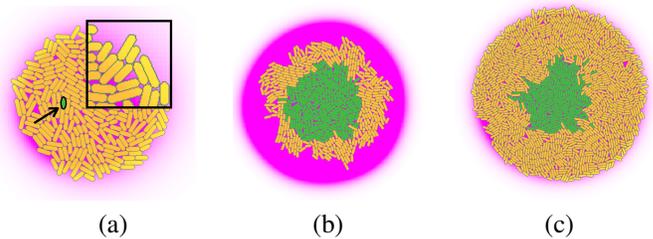


Figure 1: A two-stage homeostatic growth process. (a) A leader cell (*green*) emits a morphogen (*pink*; *inset*: zoom). Other cells (*yellow*) continually divide but die at the periphery where the morphogen concentration drops. (b,c) Second stage: a central region has differentiated into leader cells.

plicit bacterial modeling with the Gro language (Jang et al., 2012), or more abstract spatial/amorphous computing with the MGS language (Giavitto and Michel, 2002) and Proto language (Beal and Bachrach, 2006), to deal with new classes of applications characterized by the emergence of a global behavior in a large population of cells that are irregularly located and dynamically interconnected.

Morphogenetic Synthetic Biology

Current applications of synthetic biology, which focus on the individual bacterium as a chemical reactor, come from biochemistry and cellular biology. The *shape engineering* challenge of SynBioTIC belongs to “morphogenetic engineering” (ME) (Doursat et al., 2012, 2013), the transfer of natural morphogenesis to the design of the self-organizing abilities of the elements of complex systems. Generally, natural pattern formation is random and repetitive, whereas elaborate devices are the deterministic product of human design. Yet, multicellular biological organisms are striking examples of complex systems that are both entirely self-organized and strongly architectural. Accordingly, ME establishes a new object of research at the intersection between traditionally disconnected domains: it stresses the *programmability* of self-organization, underappreciated in complexity science, and, conversely, the benefits of self-organization, which are underappreciated in engineering.

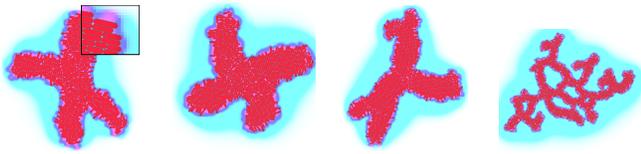


Figure 2: Multibacterial shapes obtained by varying morphogen diffusion coefficients and thresholds (*inset*: zoom).

Hand-designed virtual bacterial shapes In a first step we experimented with fundamental mechanisms that could generate collective behaviors typical of a cell assembly (including prokaryotic cells not prone to multicellularity), such as homeostasis, self-repair, and shape development. These mechanisms are described by a set of rules at the cellular level implemented in a Gro program. Fig. 1a shows an example of homeostatic process based on cell division, morphogen diffusion, and cell death, which lead to a stable structure with self-repairing abilities. The same process can be repeated recursively (Fig. 1b-c): once a first stable population is obtained, a central subpopulation differentiates into leader cells and proliferation starts again. This leads to the replication of the previous structure at a larger scale.

We also investigated a more decentralized approach without relying on leader cells (Fig. 2). In this scenario, all cells emit a slowly diffusive morphogen, and when a cell dies, it also sends a faster diffusive signal that reacts with the morphogen and degrades it. This rate difference creates a mechanism of border reinforcement. Moreover, it appears that the mechanical forces induced by bacterial contacts and proliferation also support *branching* structures (Fig. 2). This can be seen as an emergent high-level mechanism, in which local conditions drive the whole process to a specific structure.

Staged Evolutionary Engineering of Development The complexity of the design task increases tremendously when targeting more elaborate shapes than blobs. What key mechanisms should be used? What morphogen factors and chemical reactions should be involved? Faced with an infinite number of possible gene regulation and molecular signaling networks, combined with an infinite number of possible parameters and interpretations into various cell types and behaviors, the rational design attitude becomes untenable and one must resort to evolutionary computation. Yet, because real-world evolution is not driven by final cause, its virtual counterpart is also notoriously difficult to harness when exploring huge genotype spaces toward specific goals. In nature, there is no needle to be found in the proverbial haystack—only survival matters.

This is why, a contrived end justifying contrived means, evolutionary metaheuristics should not run unbridled but rather be steered by some amount of rational intervention from a human designer. The artificial evolution of morpho-

logical complex systems is known to be difficult to control, while *staging* (Bongard, 2011) and *human mediation* (Kowaliw et al., 2007) can be of help. In the bacterial shape challenge that occupies us here, we propose a hybrid methodology called staged evolutionary engineering of development (SEED): human mediation is used as a tool for exploration via an interactive evolutionary algorithm, and as a means of refining evolutionary goals between stages. Without staging, artificial evolution is unlikely to work; without human mediation, it would be unlikely to stumble again upon previously discovered and proven mechanisms.

For example, if the objective is to develop a six-armed starfish-like shape (not shown here), the design process can be achieved in two stages: first, homeostatic abilities should be acquired by a large number of individuals in the population; then, arm growth can be triggered from a few precursor cells that are led to differentiate on the periphery by reaction-diffusion. In any case, the idea behind SEED is to inject at each stage hand-designed mechanisms in the population, which are recorded in a genotype that can be played back (i.e. developed) later without intervention. If all goes well, the final stage should only consist of an easy parameter optimization—for example the spatial scale of the Turing pattern to obtain exactly six arms, not five or seven.

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