

# The Morphogen Circuit Builder & Compiler

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## 1 INTRODUCTION

Engineering cells to achieve programmed development of complex three-dimensional shapes (also known as morphogenesis) would provide a powerful means of tissue engineering for application areas such as regenerative medicine and drug development. Morphogenesis is a complex process involving cell-to-cell signaling, biomechanical forces, cell division, and differential adhesion. In order to program cells to organize themselves into the diverse types of structures seen in nature, as well as entirely novel structures, synthetic biologists need a toolkit of morphogenetic parts and means of composing those parts to produce predictable geometric forms. We have thus been investigating genetic “building blocks” for engineered morphogenesis and have developed the “Morphogen Circuit Builder,” a computational tool that allows us to explore a large space of designs that can be implemented with these parts, and a “Morphogen Compiler” that uses the circuit builder to generate a such designs from a high-level description of target morphologies.

## 2 MORPHOGEN CIRCUIT BUILDER & COMPILER

The Morphogen Circuit Builder is a high-level Java API that represents circuit designs using the Synthetic Biology Open Language format (SBOL). Leveraging SBOL allows us to compose hierarchical designs from abstract genetic modules by connecting regulatory inputs, outputs, and recombination targets. Abstract modules are then instantiated by selecting parts from an inventory of DNA parts, including promoters, regulatory elements, recombinases, recombination sites, a variety of cadherin coding sequences, reporters, and degradation tags. The circuit builder stores and accesses these virtual parts in a SynBioHub repository [3] populated by scraping GenBank sequences from vector maps and converting them to SBOL format. A key step in the process was annotating these modules with interaction information and descriptive ontology terms so that the compiler can automatically select and appropriately match modules together.

The circuit builder can also transform multi-layered, modular designs into one or more target sequences. This involves flattening the modular, hierarchical design to a single layer and unifying all input/output connections. Once this is accomplished, all of the sequences of sub-components are concatenated together into a single sequence for each plasmid to be assembled and delivered.

The circuit builder also automatically generates a two-dimensional model of morphogenetic differentiation and sorting. These models are based on the Cellular Potts Model

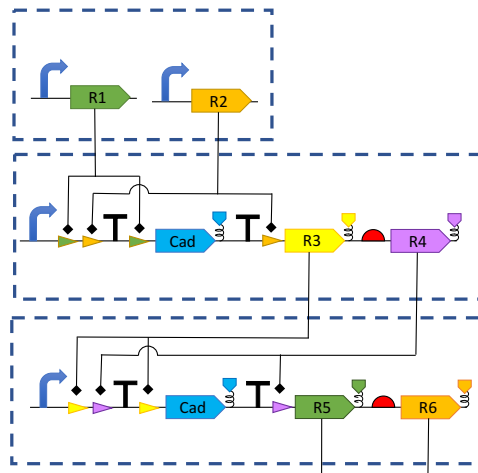


Figure 1: Switch modules can be chained in order to program multi-stage differentiation.

and can be simulated using CompuCell3D [5]. Although a variety of cell modeling frameworks could be applicable, CompuCell3D’s programmatic API enables integration with Morphogen Compiler and includes plugins for simulating different processes related to morphogenesis (e.g., diffusion gradients, division). Characteristic parameters, such as expression level or recombination efficiency, are associated with modules in the repository, which then determine the corresponding parameters in the model. During compilation, as one module is connected to another, the compiler performs operations that augment the model depending on which type of modules are being connected. Furthermore, by exploring a variety of circuit designs, we have established a predicted phase space for certain morphogenetic systems, which has then allowed us to implement the Morphogen Compiler. While the examples here are based specifically on a readily available library of parts controlling differential adhesion, our toolchain can be extended to apply to other morphogenetic processes as parts become available.

The Morphogen Compiler generates a circuit design based on a user-specified, high-level description of a morphology. The compiler is capable of generating designs for multiple types of morphologies, such as multi-layer, concentric sorted balls (analogous to gastrulation) or specialized, cell clusters interspersed through tissue (analogous to clusters of endocrine cells in pancreatic tissue) [4]. The user specifies the desired morphology and target characteristics such as diameter, layer thickness, average cluster size, and/or number of clusters. Morphologies are then generated by controlling the probability with which cells differentiate into different

types, as well expression levels of one or more types of cadherins (a family of cell-adhesion membrane proteins playing a key role in tissue morphogenesis, leading to cell sorting through differential adhesion [2]). The compiler then generates a circuit design by selecting modules with appropriate parameters, then proceeds to realization and/or simulation via the circuit builder as described above.

### 3 RESULTS

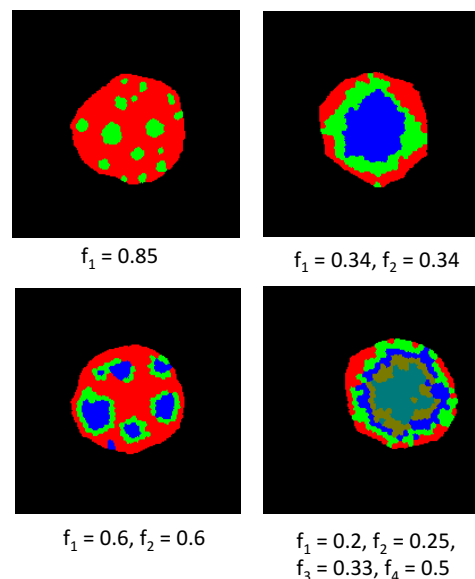
We have tested the compiler and circuit builder with a variety of differentiation cascade circuits. These cascades are composed of a modular recombinase-based switch that differentiates a region probabilistically into either of two cell fates, one of which expresses an adhesion protein while the other produces additional recombinase outputs that can then be chained to another downstream differentiation switch. This motif based design thus allows specification of structures with an arbitrary number of cell types. Figure 1 shows how a differentiation switch is implemented and how multiple switches can be chained in a cascade.

Differentiation into one cell fate versus the other is governed by competition between a pair of recombinases for their cognate recombination sites. The probability of one recombination event versus the other depends on the relative expression levels of each recombinase, which is modulated through the use of degradation tags, which have previously been demonstrated for fine-tuning circuits [1].

The differentiation switches are parameterized with a differentiation probability ( $f$ ) that determines the relative probability of selecting a lower-adhesion cell fate vs. a higher-adhesion cell fate: at each stage, low relative  $f$  will tend to result in complete cell sorting, while high  $f$  will instead tend to result in quasi-stable cluster formation. Cadherin module expression levels are then modulated to achieve the parameterized degree of adhesion means by degradation tags, thus allowing an exponential number of different morphogenetic patterns to be generated from differential tuning of a single composable motif. Figure 2 shows examples (using CompuCell3D simulation of compiler-generated models) of different morphologies that can be generated by modulation of the number of stages and the level of  $f$  for each stage.

### 4 FUTURE WORK

Our future goals are to explore a wider range of possible morphogenetic shapes by optimizing simulation parameters to better produce desired topological characteristics and match these more closely to laboratory results. To support this goal, we have implemented algorithms in the simulator that calculate image-based, topological parameters, such as cluster number, cluster size, and heterotypic boundary length (a measure of cell sorting). Additional metrics, such as image symmetry will need to be considered as well. In addition,



**Figure 2: Controlling probability of switching into cell types with different cadherin expression profiles can generate different morphologies, as shown in these CompuCell3D[5] simulations generated by the Morphogen Compiler.**

we have parallelized simulations so that we can efficiently explore large parameter spaces. These features will allow us to identify optimal parameter sets that generate a variety of target morphologies, including asymmetric morphologies.

### 5 ACKNOWLEDGEMENTS

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