A scalable pipeline for designing reconfigurable organisms

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Here, we demonstrate a scalable approach for designing living systems in silico using an evolutionary algorithm, and we show how the evolved designs can be rapidly manufactured using a cell-based construction toolkit. The approach is organized as a linear pipeline that takes as input a description of the biological building blocks to be used and the desired behavior the manufactured system should exhibit (Fig. 1). The pipeline continuously outputs performant living systems that embody that behavior in different ways. The resulting living systems are novel aggregates of cells that yield novel functions: above the cellular level, they bear little resemblance to existing organs or organisms.

Living systems are more robust, diverse, complex, and supportive of human life than any technology yet created. However, our ability to create novel lifeforms is currently limited to varying existing organisms or bioengineering organisms in vitro. Here we show a scalable pipeline for creating functional novel lifeforms: AI methods automatically design diverse candidate lifeforms in silico to perform some desired function, and transferable designs are then created using a cell-based construction toolkit to realize living systems with the predicted behaviors. Although some steps in this pipeline still require manual intervention, complete automation in future would pave the way to designing and deploying unique, bespoke living systems for a wide range of functions.

Significance

Most technologies are made from steel, concrete, chemicals, and plastics, which degrade over time and can produce harmful ecological and health side effects. It would thus be useful to build technologies using self-renewing and biocompatible materials, of which the ideal candidates are living systems themselves. Thus, we here present a method that designs completely biological machines from the ground up: computers automatically design new machines in simulation, and the best designs are then built by combining together different biological tissues. This suggests others may use this approach to design a variety of living machines to safely deliver drugs inside the human body, help with environmental remediation, or further broaden our understanding of the diverse forms and functions life may adopt.

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Data deposition: The source code necessary for reproducing the computational results reported in this paper can be found at GitHub (https://github.com/skriegman/reconfigurable_organisms).

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Fig. 1. Designing and manufacturing reconfigurable organisms. A behavioral goal (e.g., maximize displacement), along with structural building blocks [here, contractile (red) and passive (cyan) voxels], are supplied to an evolutionary algorithm. The algorithm evolves an initially random population and returns the best design that was found. The algorithm is rerun 99 times starting with different random populations, generating a diversity of performant designs in silico (A; SI Appendix, section S5). Performant designs are then filtered by their robustness to random phase modulation of their contractile cells (B; SI Appendix, section S7), constructed in vivo using developing Xenopus cardiomyocyte and epidermal cell progenitors (C–F; SI Appendix, section S8), and placed on the surface of a Petri dish where their behavior is observed and compared to the design’s predicted behavior (SI Appendix, section S9). Discrepancies between in silico and in vivo behavior are returned to the evolutionary algorithm in the form of constraints on the kinds of designs that can evolve during subsequent design-manufacture cycles (G; SI Appendix, section S6). Concurrently, tissue layering and shaping techniques are modified such that realized living systems behave more like their virtual model (SI Appendix, section S8).

As there are likely to be many differences between the simulated and targeted physical environments, performant designs are passed through a robustness filter which only allows passage of designs that sustain the desired behavior in the face of noise (SI Appendix, section S7). Previous work has shown that noise resistance in simulation is a simple and effective predictor of whether a design will maintain its behavior when instantiated physically (18).

The surviving noise-resistant designs are then passed through a build filter (SI Appendix, Fig. S4) which removes designs that are not suitable for the current build method (SI Appendix, Fig. S6) or unlikely to scale to more complex tasks in future deployments. The manufacturability of a design depends on the minimal concavity size that will persist in aggregations of developing stem cells, which tend to close small gaps in their collective geometry (SI Appendix, Fig. S7). The scalability of a design depends on its proportion of passive tissue, which provides space for future organ systems or payloads (SI Appendix, Fig. S13).

The designs that successfully pass through the build filter are then built out of living tissues. Pluripotent stem cells are first harvested from blastula stage Xenopus laevis embryos, dissociated, and pooled to achieve the desired number of cells. Following an incubation period, the aggregated tissue is then manually shaped by subtraction using a combination of microsurgery forceps and a 13-μm wire tip cautery electrode, producing a biological approximation of the simulated design. Further, contractile tissue can be layered into the organism through the harvesting and embedding of Xenopus cardiac progenitor cells, an embryonically derived cell type which naturally develops into cardiomyocytes (heart muscle) and produces contractile waves at specific locations in the resultant shaped form (SI Appendix, Fig. S6).

The final product of this procedure is a living, 3D approximation of the evolved design, which possesses the ability to self-locomote and explore an aqueous environment for a period of days or weeks without additional nutrients. These organisms are then deployed into their physical environment, and resultant behavior, if any, is observed (Fig. 3). Behaviors are then compared against those predicted by their simulated counterparts to determine whether or how well behaviors transferred from silico to vivo (Fig. 4).

After several organisms have been deployed and observed, it is likely that they exhibit varying amounts of the desired behavior. Common patterns among the successful systems are distilled down into constraints and supplied back to the evolutionary algorithm, which now evolves designs that are not just performant but also conform to the constraints (SI Appendix, section S6). This increases the success likelihood of subsequent design-to-deployment attempts.

Reconfigurable organisms were evolved to exhibit four different behaviors: locomotion, object manipulation, object transport, and collective behavior (SI Appendix, section S10). To achieve this, the pipeline was employed four times.

Locomotion. To obtain a diverse population of designs, 100 independent trials of the evolutionary algorithm were conducted (Fig. 2A–C), each starting from a different set of initial random designs. During each trial, designs were selected based on net displacement achieved during a 10-s period (with randomized, phase-modulated contraction, cycling at 2 Hz). Additional selection pressures were applied to maintain diversity by inducing competition within and between unique genetic lineages within each trial (19), yielding unique ecological dynamics (SI Appendix, section S5). The most fit designs at the end of each trial were extracted (Fig. L4) and passed through the robustness and build filters (SI Appendix, Fig. S4). During this filtering process, buildable and scalable designs that retain rapid locomotion during...
random perturbations are selected for manufacture (Fig. 3 and SI Appendix, Fig. S6).

Cilia, which produce locomotion through metachronal waves (the generation of sequential and directional propagating waves, as opposed to synchronized beating), were not modeled in silico and were suppressed in vivo through embryonic microinjection of mRNA transcribing the Notch intracellular domain (Notch ICD) (20). Thus, any displacement results from contractile cardiac muscle tissue that pushes against the surface of the dish. This simplifies the simulation and its comparison to the realized organism. Trajectories of deciliated designs are compared in silico and in vivo, in two orientations (upright and inverted 180° about the transverse plane) thus isolating the impact of the designed morphology on the difference between predicted and realized behavior. For at least one design, the data suggest that the desired behavior successfully transferred when it was upright but not when inverted (Fig. 4). More specifically, the upright organisms’ direction of movement matched that of the in silico design under random perturbations ($P < 0.01$; details in SI Appendix, section S9), and inverting the design significantly reduced its net displacement both in silico ($P < 0.001$) and in vivo ($P < 0.0001$). This suggests that successful transference did not result by chance but rather was due to the design itself.

Object Manipulation. When the environment is strewn with particulate matter, motile designs spontaneously aggregate the external objects both in silico (SI Appendix, Fig. S10) and in vivo (Fig. 3F and SI Appendix, Fig. S11). More precise object manipulation can be selected for an explicit goal, such as specifying target areas from which debris should be cleared, or target objects to discard. The latter goal was implemented and primitive end-effectors evolved in simulation (SI Appendix, Fig. S12).

Object Transport. Some designs evolved for displacement reduced hydrodynamic drag (SI Appendix, section S6) via a hole through the center of their transverse plane. This more complex topology was realized in vivo (SI Appendix, Fig. S13) but was not layered with contractile tissue. In simulation, this emergent feature can be exapted as a pouch to store and transport objects. In a subsequent round of evolution, pouches were explicitly incorporated as a design constraint, and the new goal of maximizing the distance of the carried object was employed. This yielded evolved object transport in silico (SI Appendix, Fig. S13).

Collective Behavior. Multiple designs can be placed in the same environment, yielding collective behavior (21) (SI Appendix, Figs. S10 and S11). Several such behaviors predicted in silico were observed in vivo. For instance, two designs often collide, form a temporary mechanical bond, and orbit about each other for several revolutions before detaching along tangential trajectories (SI Appendix, Fig. S10). This phenomenon is more pronounced when cilia are not inhibited on the organisms: individuals frequently become entangled with their neighbors, often changing partners across an observation (Fig. 3F and SI Appendix, Fig. S11).

Discussion

Although simulation and design of rigid structures and machines has been possible for some time, only recently has it become computationally tractable to simulate the combined behavior of arbitrary aggregates of soft components with differing material and actuation properties (22). As shown here, such fine-grained simulations can be embedded in evolutionary search methods to discover designs that can be instantiated in biological rather than artificial materials.

The resulting organisms embodied not only the structure (SI Appendix, Fig. S8) of evolved in silico designs but also their behavior...
Fig. 3. Manufacturing reconfigurable organisms. (A) Aggregation of pluripotent blastula cells harvested from *X. laevis* embryos. (B) Shaping results in 3D representations of the evolved in silico designs. (C) Layering of cardiac progenitor cells results in contractile cardiomyocyte tissue at specific locations, visualized by red fluorescent lineage tracer. (D) Time-lapse imaging of self-locomotion in an aqueous environment. (E) Emergent behavior of debris aggregation by an individual within the environment and (F) by groups of reconfigurable organisms over a 24-h period (*SI Appendix*, section S10.4). (Scale bars: 500 μm for A–E and 5 mm for F, respectively.)
The organisms D). Six reconfigurable organisms were built which embodied this design (e.g., B) (SI Appendix, section S9). Three were evaluated four times and the other three were evaluated five times for 10 min each (27 blue curves in C). The organisms’ direction of movement matched the design’s predicted direction of movement ($P < 0.01$; details in SI Appendix, section S9). To determine whether the organisms’ movement was a result of chance or due to the design’s evolved geometry and tissue placement, geometry and tissue distribution was altered by rotating the design 180° about its transverse plane (D) and evaluating it another 25 times in silico (pink curves in F). Each of the six organisms were likewise inverted (E): four were evaluated five times while the remaining two were only evaluated once (22 blue curves in F). Inverting the design significantly reduces its net displacement ($P < 0.001$), as did inverting the organisms ($P < 0.0001$).

Beyond the applications reported here, the generality of this approach is as of yet unknown. But, advances in machine learning, soft body simulation, and bioprinting are likely to broaden the potential applications to which it may be put in the future. Applications could be numerous, given the ease of misexpressing novel proteins and synthetic biology pathways and computational circuits in *Xenopus* cells (27). Given their nontoxicity and self-limiting lifespan, they could serve as a novel vehicle for intelligent drug delivery (28) or internal surgery (29). If equipped to express signaling circuits and proteins for enzymatic, sensory (receptor), and mechanical deformation functions, they could seek out and digest toxic or waste products, or identify molecules of interest in environments physically inaccessible to robots. If equipped with reproductive systems (by exploiting endogenous regenerative mechanisms such as occurs in planarian fissioning), they may be capable of doing so at scale. In biomedical settings, one could envision such biobots (made from the patient’s own cells) removing plaque from artery walls, identifying cancer, or settling down to differentiate or control events in locations of disease. A beneficial safety feature of such constructions is that in the absence of specific metabolic engineering, they have a naturally limited lifespan.

These methods, reagents, and data extend the breadth of model organisms available for study by designing living systems that are as orthogonal as possible to existing species, yet capable of being built from existing cell types. By enabling a computationally guided interplay between emergent and designed processes, this platform facilitates studies of the relationship between genomes
(in our case, wild-type *X. laevis*), the resulting body plan, and its behaviors in diverse environments. Thus, reconfigurable organisms could serve as a unique model system facilitating work in the evolution of multicellularity, exobiology, artificial life, basal cognition, and regenerative medicine. If equipped with electrically active cells and selected for cognitive or computational functions (30), such designed systems may similarly broaden our understanding of how intelligence can be instantiated in living as well as nonliving systems.

**Materials and Methods**

**Evolutionary Design.** Designs (SI Appendix, section S2) were evolved inside a physics engine (SI Appendix, section S3) as reconfigurable aggregations of passive and contractile voxels (Fig. 1). On the first pass through the pipeline using the goal behavior of locomotion, we simulated designs on land and allowed the evolutionary process to finely tune their actuation. This resulted in highly performant but nontransferable designs (SI Appendix, Fig. S2) with powerful, bounding gaits that are not obtainable in vivo with the current build method (SI Appendix, section S8). These gaits were characterized by timeframes (on average, 47% of the gait cycle) in which no part of the silico design was in contact with the simulated ground plane. In vivo, however, the deciliated organisms always kept part of their ventral surfaces in contact with the surface of the dish due to negative buoyancy.

These discrepancies were rectified by adding constraints into the pipeline in the form of adjustments to environmental and actuation settings, which were altered as follows. On the second pass, the fidelity of the simulated environment was increased by incorporating first-order hydrodynamics: the modified environment consisted of an infinite plane submerged in water, which was approximated by decreasing the coefficient of gravitational acceleration (increasing buoyancy) and applying a drag force to each voxel face on the design’s surface (SI Appendix, section S6).

Secondly, actuation was randomized: contractile cells were revised to have random phase offsets from a central pattern generator (a sine wave with frequency 2 Hz). More specifically, each voxel of a randomly configured design (one of which was injected into the population at each generation; SI Appendix, section S3) was assigned a random phase offset, which was held fixed in its descendants (the entire clade). Mutations switched each voxel to be either present or absent, and, if present, either passive or active (contractile), but the original phase offset, at every location in the workspace, was hardcoded. This reduced the dependence on precisely timed excitation, and promoted the discovery of more robust mechanical structures (SI Appendix, Fig. S3).

The behavior of designs generated on the second pass better matched the behavior of the actual living systems; on average, designs were in contact with the ground plane for 93.3% of their evaluation period, compared to just 52.7% on the first pass (SI Appendix, section S6).

**Robustness Filter.** The most performant designs (Fig. 1A) were sorted by their robustness to random perturbations in their actuation. Phase offsets stored in the genotype were mutated by adding a number that was drawn randomly from a normal distribution with mean zero and SD [−π/2 to π/2] range of valid phase-offset values). This hyperparameter was selected to be large enough to scramble the original phase-offset value without allowing the evolutionary process to finely tune their actuation. This resulted in highly performant but nontransferable designs (SI Appendix, Fig. S2) with powerful, bounding gaits that are not obtainable in vivo with the current build method (SI Appendix, section S8). These gaits were characterized by timeframes (on average, 47% of the gait cycle) in which no part of the silico design was in contact with the simulated ground plane. In vivo, however, the deciliated organisms always kept part of their ventral surfaces in contact with the surface of the dish due to negative buoyancy.

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**Build Filter.** The most robust designs are evaluated by their manufacturability under the current build method, which layers contiguous tissue regions sequentially (SI Appendix, Fig. S6). The minimal concavity was examined by adding constraints into the pipeline in the form of adjustments to environmental and actuation settings, which were altered as follows. On the second pass, the fidelity of the simulated environment was increased by incorporating first-order hydrodynamics: the modified environment consisted of an infinite plane submerged in water, which was approximated by decreasing the coefficient of gravitational acceleration (increasing buoyancy) and applying a drag force to each voxel face on the design’s surface (SI Appendix, section S6).

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**Realizability Generator.** Reconfigurable organisms were created using *Xenopus* embryos as donor tissue under methods approved by the Institutional Animal Care and Use Committee of the University of Washington, Department of Laboratory Animal Medicine under protocol number M2017-53.

Fertilized *X. laevis* eggs were reared in a 0.1x, pH 7.8, Marc’s Modified Ringers solution (MMR) using standard protocols and staged according to Nieuwkoop and Faber (32, 33). For shaping experiments, animal caps were manually cut at St. 9 using surgery forceps (Dumont, 11241–30 #4) and transferred to calcium- and magnesium-free medium for 5 min (50.3 mM NaCl, 0.7 mM KCl, 9.2 mM Na2HPO4, 0.5 mM KH2PO4, 2.4 mM NaHCO3, 1.0 mM edetic acid [EDTA], pH 7.3). The outer ectoderm layer was manually removed and discarded, while the inner layer was agitated until fully dissociated (cells are this stage are largely pluripotent, but differentiate into ectoderm without further intervention). Material from five animal caps was pooled and transferred to a welled dish containing 0.75x MMR. After 24 h at 14 °C, the spherical reaggeregate was moved to a clean 1% agarose-coated dish containing 10 mL 0.75x MMR and 5 μl gentamycin (ThermoFisher Scientific, 15710072). Forty-eight hours after tissue regeneration the resulting tissue (now fated to become specific epidermal cell lineages including ionocytes, small secretory cells, and goblet cells), was shaped using a combination of microsurgery forceps and a MC-2010 microactuary instrument with 13-μm wire electrodes (Protech International, 13-μm wire tip curvature the second) which was reseeded as necessary for 3 h to create the desired anatomical outcome, after which it was moved to a clean 1% agarose-coated dish containing 10 mL 0.75x MMR and 5 μl gentamycin and raised at 14 °C.

For contractile movement experiments, cohorts of *Xenopus* embryos were microinjected with one of two synthetic mRNAs at the four-cell stage using standard protocols (34). mRNA for its fluorescent lineage tracer tdTomato (34) and the multicellular inhibitor Notch ICD (20, 35) was synthesized using mMESSAGE transcription kits (ThermoFisher Scientific, AM1340). Injections were performed in 3% Ficoll solution using a pulled capillary to deliver 370 pg of mRNA for each transcript to all four cells. tdTomato microinjected embryos were reared for 22 °C while Notch ICD injected embryos were reared at 14 °C. Twenty-four hours after injection, stage 10 Notch ICD injected embryos were moved to a 1% agarose-coated Petri dish containing 0.75x MMR, and animal caps were manually cut using surgery forceps as above. In addition, stage 23–24 tdTomato injected embryos were transferred to the same dish and the presumptive heart field was excised with the outer layer of ectoderm then removed and discarded. Presumptive heart tissue was then placed between two Notch ICD injected animal caps, and the three layers were allowed to heal for 1 h at 22 °C. Following healing, the tissue was moved to clean 1% agarose-coated dish containing 10 mL 0.75x MMR and 5 μl gentamycin and raised at 14 °C. For shaping, resultant tissue was sculpted as above using a combination of microsurgery forceps and a MC-2010 microactuary instrument.

**Transferability Filter.** All samples were imaged live in 0.75x MMR at 20 °C using a Nikon SMZ-1500 microscope equipped with both top and bottom plane illumination. Still images were captured on a Coolimaging Bega 2000R CCD camera and videos were captured using a Sony IMX234 at a sample rate of 30 frames per second. XY movement tracks were extracted for each run using Noldus Ethovision 14 software, and smoothed using a one-dimensional Gaussian filter (SI Appendix, section S9.1). The tdTomato lineage tracer was imaged using a standard tetramethylrhodamine isothiocyanate (TRITC) filter cube and fluorescent light source to verify cardiac muscle cell location, and GFP signal was imaged with a standard fluorescein isothiocyanate (FITC) filter cube to verify epidermal cell location (SI Appendix, section S9.2).

**Data Availability.** The source code necessary for reproducing the computational results reported in this paper can be found at GitHub (36).

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