Dataset 2. Examples of handcrafted design spaces

A class of natural systems. Elementary gene circuits provide an example of a molecular system with a specific design for a particular function. These circuits involve a set of genes coding for a specific effector function and a gene encoding their specific regulator protein. Regulator and effector gene expression in bacteria have been found to exhibit two extreme forms of coupling: complete uncoupling (e.g., effector genes are induced with no change in the expression of the regulator gene), and perfect coupling (e.g., regulator and effector genes are co-induced to the same level). Is there a rule governing selection of the coupling pattern in a particular context? The answer is yes (1).

A simplified kinetic model of an inducible catabolic system capable of exhibiting the extreme forms of coupling is shown in Figure S2A; the corresponding design space is shown in Figure S2B. The vertical axis represents the kinetic order for the influence of the inducer on the rate of effector gene transcription (green). The horizontal axis represents the kinetic order for the influence of the regulator on the rate of effector gene transcription (red); a positive value corresponds to the influence of an autoregulated activator protein, a negative value to that of an autoregulated repressor protein, and a value of zero to the completely uncoupled case in which the influence of the regulator is unchanging.

The “box” in this space represents the physical limits on the magnitude of these kinetic orders, which is determined by the number of binding sites involved in the
**Figure S2.** Coupling of expression in elementary gene circuits. (A) Kinetic model of an inducible catabolic system with an autoregulated activator protein (+), or an autoregulated repressor protein (-), or a completely uncoupled regulator (0). (B) Design space exhibiting mode of molecular control (activator or repressor), physical bounds (limiting box for the kinetic orders), local stability boundary, and capacity for expression (low, intermediate, high) [see (1) for details].

control. These are small integer values, typically 2 or 4, but the exact values do not change the qualitative results. The inclined lines radiating from the fixed point on the horizontal axis are functional constraints that represent the locus of systems having the same capacity for regulation (the ratio of maximal to basal level of expression); the steeper the slope, the greater the capacity. The dashed vertical line, with the same fixed point on the horizontal axis, represents the boundary of local instability for the case in
which the rates of production and loss of the natural inducer are equally inducible. The slope of the dashed line becomes positive if there is a contribution to the production rate that is not inducible; it becomes negative if there is a contribution to the loss rate that is not inducible. As the slope becomes increasingly negative, an otherwise stable system with a graded response to substrate becomes an unstable system with an all-or-none hysteretic response (2).

The alternative forms of coupling were compared on the basis of several quantitative criteria for functional effectiveness. The results can be summarized as follows. The performance of a system improves as its representative point moves to the left in the design space. Thus, for systems with repressor control and a low capacity for regulation, one predicts perfect coupling. In the limiting case of a high capacity, the only option is complete uncoupling. Conversely, for systems with activator control and a low to high capacity for regulation, one predicts complete uncoupling. In the case of capacities even higher than allowed for systems with repressor control, the only option is perfect coupling.

Essentially the same results are obtained when one considers a more general class of circuitry (3) in which regulator and effector gene expression can exhibit one of three types of coupling: directly coupled (e.g., induction of both); inversely coupled (e.g., induction of effector genes and repression of regulator gene); and uncoupled (e.g., effector genes are induced with no change in the expression of the regulator gene).

It is important to note that many of the results are independent of the specific values for the parameters and depend only on the topology of the circuitry and the signs associated with the various interactions. Thus, these qualitative results are independent
of minor genetic changes that would affect the numerical values of the parameters. Modifications of the circuit topology and the signs of the interactions would require significant genetic changes. These predictions of coupling are supported by experimental data for more than 50 different systems (4) and may be viewed as establishing a design principle for this large class of inducible catabolic systems.

**A class of engineered systems.** Elementary gene circuits also have been reconfigured with the aim of achieving an engineered objective. An example involving components of the lactose and nitrogen circuits of *Escherichia coli* is shown in Figure S3A (5). In this case, the goal was to engineer a sustained oscillator. The design space for this system (Figure S3B) and the predicted behaviors are essentially identical to those for repressible biosynthetic operons with a perfectly coupled activator (6). The horizontal axis represents the kinetic order for the influence of the activator on the rate of activator gene transcription (green). The vertical axis represents the product of two kinetic orders; one represents the influence of the repressor on the rate of activator gene transcription (red), whereas the other represents the influence of the activator on the rate of repressor gene transcription (green). Again, there is a “box” that represents the physical limits on the magnitude of the kinetic orders, which is determined by the number of binding sites involved in the control. These are small integer values, typically 2 to 4 for the horizontal axis and -4 to -16 for the vertical axis, but the exact values do not change the qualitative results. The inclined lines represent the boundaries for local instability: between these the system exhibits locally-stable behavior (region 1), above the line with negative slope the system exhibits hysteretic-switch behavior (regions 2 and 3), and below the two lines
Figure S3. Engineering of elementary gene circuits. (A) Kinetic model involving components from the nitrogen (positive: green) and lactose (negative: red) control systems. (B) Design space exhibiting modes of molecular control, physical bounds, local stability boundaries [see (5) for details].

the system exhibits limit-cycle behavior (region 4). Thus, the design space is partitioned into three phenotypically distinct regions.

Preliminary experimental results with the construct modeled in Figure S3A exhibited damped oscillations, indicating that the point representing the system in design space lies within region 1 (Figure S3B). Furthermore, a rough location for the system’s point could be predicted by estimating the parameter values from the experimental data. Since all the labeled landmarks in the design space are well characterized, one can calculate the shortest distance that the system’s point must move in order to cross into region 4 (or that the boundaries must move to cross the system’s point and leave it in
region 4), thereby signaling the change to limit-cycle behavior. The results predict that changes in some parameter values move the system to limit-cycle behavior whereas others do not (Figure S4). Changes in the values of the parameters associated with the regulatory interactions provide the most effective route to achieving limit-cycle behavior; however, these changes are the more difficult to implement experimentally. Experiments

![Figure S4](image)

**Figure S4.** Tolerance to variation in values of parameters that would allow the system to transition from region 1 to region 4 in the design space of Figure S3B. The distance to the boundary between regions 1 and 4, d, is plotted as a function of the rate constant for degradation, β. Each is normalized with respect to its value at the nominal operating point in region 1. (A) Changes in the rate constant of activator mRNA cannot induce the system to leave region 1. (B) Changes in the rate constant of the repressor mRNA can cause the system to leave region 1 (decrease the distance to the boundary, d, to zero, or minus infinity in the log plot) when its value is decreased five fold.

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designed to test other parameter changes, which were predicted to produce sustained oscillation, were only partially successful [see (5) for details]. Other investigators (7) have used different constructs to engineer sustained oscillators.

References


