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Recognizing and engineering digital-like logic gates and switches in gene regulatory networks

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A central aim of synthetic biology is to build organisms that can perform useful activities in response to specified conditions. The digital computing paradigm which has proved so successful in electrical engineering is being mapped to synthetic biological systems to allow them to make such decisions. However, stochastic molecular processes have graded input-output functions, thus, bioengineers must select those with desirable characteristics and refine their transfer functions to build logic gates with digital-like switching behaviour. Recent efforts in genome mining and the development of programmable RNA-based switches, especially CRISPRi, have greatly increased the number of parts available to synthetic biologists. Improvements to the digital characteristics of these parts are required to enable robust predictable design of deeply layered logic circuits.

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Introduction

Electronic computers contain powerful decision-making circuits, built using switches with well-defined digital characteristics that are connected to produce Boolean logic operators. Synthetic biologists are making progress at replicating digital decision making in living organisms, aiming to program cells for applications in areas such as environmental sensing and medicine [1–3].

Digital-like behaviour in natural and synthetic biological systems is used to produce in effect all-or-nothing responses: the output signal from digital-like modules switches between low and high output levels (OFF/ON; binary 0/1) over a short range of input signal. Biology is inherently analogue due to the stochastic nature of the molecular interactions that propagate information flow, and so biological switches possess digital characteristics to greater or lesser degrees. Strongly digital-like characteristics are desirable when implementing biological switches in bio-computing circuits as Boolean logic gates (Figure 1a). A steep, ultrasensitive transition between OFF and ON states is key, minimising signal degradation when logic gates are layered (a condition where the output of one logic gates acts as the input for another) [4••]. A large difference between output levels in the OFF and ON states also reduces noise propagation through the circuit, maintaining signal fidelity.

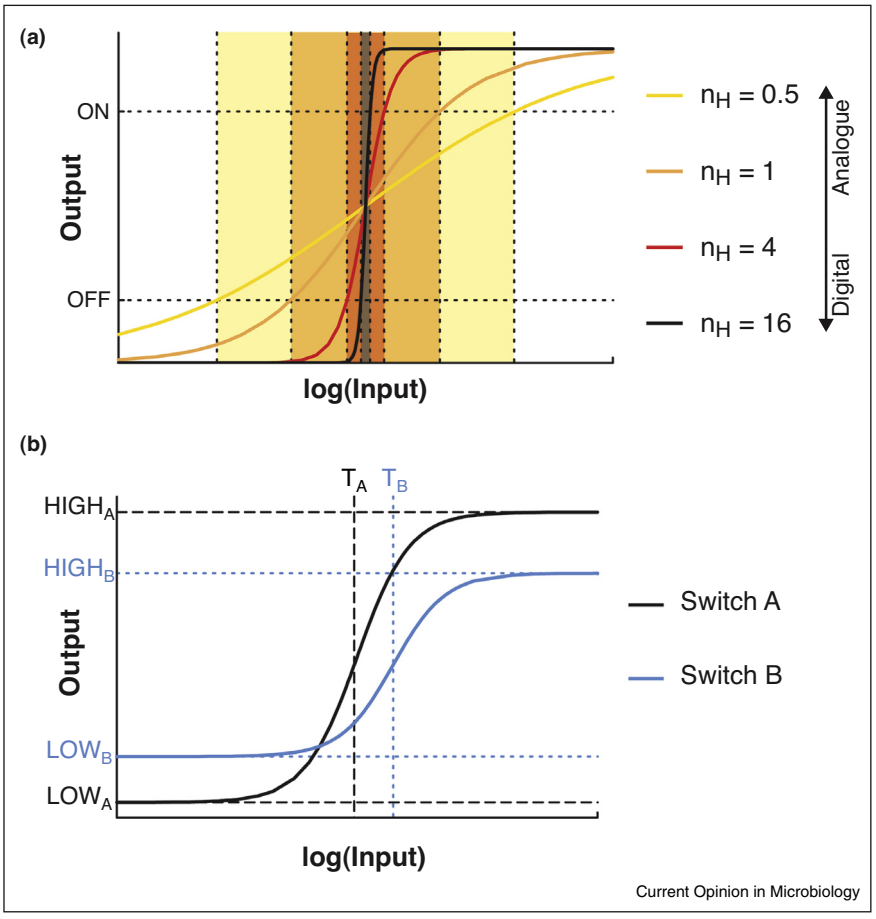
The inputs and outputs from connected gates in a circuit must be composable both in terms of signal type — so information can be transferred — and amplitude — so that the OFF and ON output levels of an upstream gate are below and above the switching threshold for the downstream gate (Figure 1b). Ideally the switching threshold and output level of a gate should be tunable. Decision-making also requires that logic gates receive inputs from multiple upstream gates, whilst remaining orthogonal to signals from all other host and synthetic components in the system [5,6].

Here we review efforts that have been made to identify parts for digital bio-computation, with an emphasis on large part families and those that are amenable to rational redesign, as these will form the basis of future large-scale genetic logic circuits. Improvements to the digital characteristics of existing biological logic gates are necessary to maintain signal fidelity in deeply layered circuits, and we discuss engineering strategies for making these enhancements.

Identifying modules with digital characteristics

Characterisation of a component's switching properties allows key properties such as dynamic range, activation threshold, and transfer function steepness to be determined [7•,8]. The nonlinear, ultrasensitive response to an input signal that characterises digital-like biological parts is usually quantified by fitting the Hill function to the curve, with ultrasensitive mechanisms having an apparent Hill coefficient greater than one [9]. Fundamental knowledge of a biological part's mechanisms of action allows

Figure 1



Digital-like behaviour in biological signal processing. (**a**) Biological switches may be more or less digital-like in character, depending on the ultrasensitivity of the switching mechanism. Hill function curves with different Hill coefficients (n_H; curves with $n_H > 1$ display a cooperative, ultrasensitive response) are shown. The switching range between arbitrary OFF and ON output thresholds (dashed horizontal lines) decreases (i.e. becomes more digital-like) with increasing ultrasensitivity. (**b**) Two example curves for switches with different properties are shown: switch A (black) has a lower activation threshold and larger output dynamic range compared to switch B (blue). The lower OFF state and higher ON state of switch A means it can connect effectively with downstream gates that possess a wider range of activation thresholds and broader switching profiles compared to switch B. T_A/T_B: activation threshold for switch A/B, arbitrarily defined as input required for 50% output; $LOW_{A/B}$, $HIGH_{A/B}$: minimum and maximum output levels for switch A/B.

probable candidates for logic gates to be selected: Components with known cooperative mechanisms, such as the TetR repressor's ligand-induced weakening of DNA binding affinity [10], can be chosen to provide sensitive switching; High ON:OFF ratios can be found in part types with low intrinsic leakiness, for example when a part is absolutely required for output such as a phage RNA polymerase [11]; The requirement for integration of multiple signals can be fulfilled by choosing components with activating or repressing partners, for example transcription factors which need activating chaperones [12]. Our lab has investigated the *Pseudomonas syringae* hypersensitive response pathway regulatory components as a model for engineering orthogonal digital-like control of transcription in *Escherichia coli* [2,5,13,14••] (Figure 2). A great number of similar regulatory modules exist in many different bacterial species, offering a largely untapped resource to construct versatile orthogonal genetic logic devices.

Sophisticated digital genetic circuits require a large number of composable parts that act with minimal crosstalk and cause low toxicity to the host. Genomic mining strategies can be employed to screen for orthogonal homologs of useful parts. Stanton *et al.* produced a set of 16 orthogonal TetR repressor homologs and cognate operators which was used to build NOT and NOR gates [15] (NOR gates are desirable because they are functionally complete). Whilst the design of a single repressor binding site within a strong constitutive promoter was