Design of Biological Circuits Using Signal-to-Noise Ratio

Jacob Beal
Raytheon BBN Technologies
10 Moulton Street
Cambridge, MA, USA 02138
jakebeal@bbn.com

1. MOTIVATION

Biological computing circuits have a role to play in many synthetic biology applications, such as precision cancer therapy, sensing chemical threats, or control of biosynthesis processes. Actually realizing such circuits effectively, however, has been quite difficult: until recently, neither high-precision prediction nor high-performance component libraries were available. Thus, although many design approaches for selecting components to realize a circuit have been proposed (e.g., [11, 6, 9], to name a few), it has been unclear which, if any, of these approaches was likely to actually be practical for the realization of biological circuits.

Recently, however, significant progress has been made in both circuit prediction and device performance. Calibrated flow cytometry [3] has enabled high-precision prediction of cascades and feed-forward circuits [5], as well as precision-design of resource competition systems [2]. At the same time, extensible families of high-performance devices have been created using four different architectures: TetR homologs [10], invertase logic [4], CRISPR-based repressors [7], and TALE-based repressors [8, 5].

Unfortunately, a signal-to-noise ratio (SNR) analysis of the actual properties of these device families shows that they do not yet correspond well with some of the digital logic assumptions that prior work on design approaches has relied upon. Instead, biological circuit design requires an approach that explicitly takes into account the degradation of a signal by each device in a computation, at least with the current families of available devices.

2. SIGNAL-TO-NOISE RATIO

From its inception, much of the work on biological circuits has embraced a digital logic paradigm. Key to realizing digital logic is for the amount of noise in the signal to improve from the inputs to the outputs of a device (generally via strong amplification). The amount of noise reduction—the "noise margin"—then determines the amount of noise that can be tolerated at each stage of computation without impacting the outcome of a computation of arbitrary complexity. Design tools for selecting devices to realize a circuit, such as MatchMaker [11] and SBROME [6], typically assume that there are devices available that provide noise reduction, and then attempt to select an appropriate set of such devices to realize the circuit.

We need to consider, however, whether such an assumption can actually be warranted. Mathematically, the relationship between signal and noise can be expressed as a signal-to-noise ratio (SNR), which may be computed using the standard formula: $SNR_{dB} = 20 \log_{10} \frac{A_{signal}}{A_{noise}}$ where A is the root-mean-square (RMS) amplitude of the signal and noise waveforms respectively. The efficacy of a logic device may then be expressed in terms of the difference between output and input SNR: $\Delta SNR_{dB} = SNR_{dB,out} - SNR_{dB,in}$ Any device with a significantly positive ΔSNR_{dB} can be used effectively to implement digital circuits; any other device degrades the signal that passes through it, limiting what computations are possible to implement. Moreover, the ΔSNR_{dB} that can actually be realized for a device depends on the levels and distributions of the inputs with which it is provided: a device that is positive when provided with well-matched inputs may be very negative when its inputs are instead too low or too high.

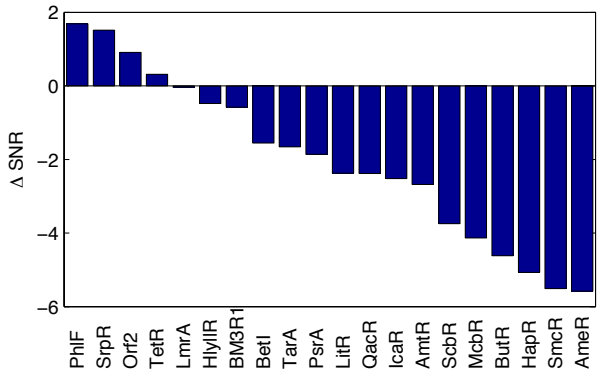

Figure 1: TetR homologs are the current best-performing logic device architecture: a few have positive maximum ΔSNR_{dB}, but most do not, and input/output levels do not generally match well between devices. Data reproduced from [1]

Characterization of synthetic biology devices and computations to date, however, has generally not actually analyzed signal to noise ratio, but instead provided only partial information, such as the ratio between "on" and "off" states, or the amplification of the device. While strong on/off ratio and strong amplification are generally necessary for good devices, they are not sufficient.

In fact, an SNR analysis of each of the current extensible high-performance device architectures, carried out in [1], reveals that none of them is currently known to be sufficient to implement complex digital logic circuits: TetR homologs [10]

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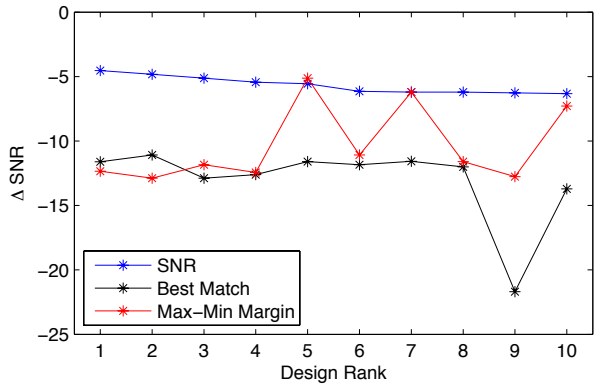

Figure 2: Design of a three-stage repressor cascade using TetR homolog devices shows that metrics based on digital assumptions are not effective at predicting signal degradation.

are currently the best-performing architecture, with a few devices providing the desired positive ΔSNR_{dB} for a narrow band of input values (Figure 1). They are highly heterogeneous, however, with most performing much more poorly, and generally poor matches between input and output levels. Invertase logic [4] has a sufficiently strong amplification, but its SNR performance is degraded by a significant non-responsive population. TALE-based repressors [8, 5] have insufficient amplification to support noise restoration. CRISPR-based repressors [7] may be better, but have only been characterized for on/off ratio, so amplification and input/output matching cannot yet be analyzed.

The effects of this insufficient ΔSNR_{dB} can be directly observed in the results reported for circuits constructed with these architectures. In every case [10, 5, 8, 7] the on/off ratio of the circuit output is much less than the on/off ratio of its inputs and earlier stages. This is symptomatic of a negative ΔSNR_{dB}, indicates that only simple and shallow circuits can currently be realized, and also indicates that digital logic noise restoration cannot be safely assumed.

3. SNR-BASED CIRCUIT DESIGN

Given the signal degradation of current biological computing devices, how do proposed approaches to design need to be adjusted? One option, of course, is to change nothing about design and just wait for devices with better ΔSNR_{dB}, but it is unclear how long this will take or to what degree it is even possible for large families of devices. More to the point, a great deal of interesting circuits can be implemented even with degrading signal strength, as is well demonstrated by the circuits in the same publications cited above.

To make principled decisions regarding the design of such circuits, we need a better metric that does not assume digital behavior. A reasonable choice for such a metric, of course, is simply SNR, since this directly measures the distinguishability of circuit outputs. For small circuits and libraries, this metric can be applied by brute force simulation of distributions. For example, Figure 2 shows the ΔSNR_{dB} ratings of the best ten designs for a three-stage inverter chain designed with TetR homologs from [10], beginning with an initial strong signal of low $10^{-1.5}$ and high $10^{1.5}$ a.u. and assuming a 2-fold standard deviation of per-cell expression. Some of the choices are actually quite non-intuitive: for example, the top ten circuits include use of HlyllR, BM3R1, and PsrA, and the strongest repressor (PhlF) appears to be a poor choice for the first inverter, with the best circuit starting with PhlF being only -9.79 dB. Heuristics based on digital assumptions, however, such as maximizing the minimum noise margin [11] (using thresholds set at the 1:1 log slope), or maximizing input/output match quality, fail to accurately predict circuit performance and may select highly sub-optimal circuits.

Therefore, in order to realize effective biological circuit design using current signal-degrading devices, we can see that is it important to take the distribution of variation into account using a metric such as SNR. Heuristic and dynamic programming techniques that work for other metrics are likely to be adaptable for SNR as well, and there is also a considerable literature from the signal processing community that may be investigated for adaptability to the biological domain as well.

4. REFERENCES

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